



Protocol

Assessing the effectiveness of targeted active case detection among high-risk populations in Southern Lao PDR

Partnership of Center for Malariology, Parasitology and Entomology, National
Institute of Public Health, and University of California, San Francisco



University of California
San Francisco

Funded by: Bill & Melinda Gates Foundation

**Version Number: 4
November 05, 2017**

Principal Investigators

<p>Adam Bennett, PhD MEI Programmatic Lead</p> <p>Malaria Elimination Initiative, Global Health Group University of California, San Francisco Phone: 1-415-476-5590 Adam.Bennett@ucsf.edu</p>	<p>Rattanaxay Phetsouvanh, MD, MSc Director</p> <p>Department of Communicable Disease Control Ministry of Health Vientiane, Lao PDR bphommasack@gmail.com</p>	<p>Andrew Lover, MS, MPH, PhD GMS Technical Program Coordinator</p> <p>Malaria Elimination Initiative, Global Health Group University of California, San Francisco andrew.lover@ucsf.edu</p>
--	--	---

Co-investigators and Key Personnel

<p>Emily Dantzer, MHS Program Manager</p> <p>Lao PDR Malaria Elimination Research Partnership Global Health Group / UCSF Global Health Sciences Vientiane, Lao PDR emily.dantzer@ucsf.edu</p>	<p>Bouasy Hongvanthong, MD Director</p> <p>Center of Malariology, Parasitology, Entomology (CMPE) Vientiane, Lao PDR cmpelao@gmail.com</p>	<p>Vatthana Nanthana, MBA Local Study Coordinator</p> <p>Lao PDR Malaria Elimination Research Partnership Global Health Group / UCSF Global Health Sciences Vientiane, Lao PDR vnanthana@gmail.com</p>
<p>NIOPH: Dr. Souraxay Phommala, DG of NIOPH</p>	<p>NIOPH: Dr. Khampheng Phongluxa, Head of Health Research Outcome Division</p>	<p>Jennifer L Smith, MSc, PhD Assistant Professor</p> <p>Malaria Elimination Initiative, Global Health Group University of California, San Francisco Jennifer.Smith@ucsf.edu</p>
<p>Jimee Hwang MD, MPH Medical Epidemiologist</p> <p>US Center for Disease Control & Prevention Center for Global Health/DPDM Malaria Branch/US President's Malaria Initiative, and Malaria Elimination Initiative, Global Health Group University of California, San Francisco</p>	<p>Sophia Hocini, MPH Surveillance Research Analyst</p> <p>Malaria Elimination Initiative, Global Health Group University of California, San Francisco Sophia.Hocini@ucsf.edu</p>	<p>Francois Rerolle, MS Geospatial specialist; PhD student</p> <p>Malaria Elimination Initiative, Global Health Group University of California, San Francisco Francois.Rerolle@ucsf.edu</p>

Jimee.Hwang@ucsf.edu		
<p>Roly Gosling, MD PhD Associate Professor</p> <p>Lead, Malaria Elimination Initiative, Global Health Group University of California, San Francisco Roly.gosling@ucsf.edu</p>	<p>Joshua Yukich, PhD Assistant Professor</p> <p>Tulane University School of Public Health and Tropical Medicine jyukich@tulane.edu</p>	<p>Bryan Greenhouse, MD, MA Assistant Professor Dept. of Medicine / Div. of Infectious Diseases University of California, San Francisco bryan.greenhouse@ucsf.edu</p>
<p>Jerry Jacobson Independent consultant</p> <p>jerryojacobson@gmail.com</p>		

Table of Contents

1	PROTOCOL SUMMARY	7
2	BACKGROUND AND INTRODUCTION	9
2.1	LITERATURE REVIEW	9
2.1.1	ACTIVE SURVEILLANCE FOR MALARIA ELIMINATION	9
2.1.2	ACTIVE SURVEILLANCE FOR MALARIA ELIMINATION IN HIGH-RISK POPULATIONS	9
2.1.3	RATIONALE	10
2.2	SIGNIFICANCE	12
2.3	PRIMARY AIM	12
2.4	PRIMARY RESEARCH QUESTIONS AND HYPOTHESES	12
2.5	STUDY OVERVIEW	13
2.6	SUMMARY OF ETHICAL ISSUES	13
3	METHODOLOGY	14
3.1	STUDY SITE	14
3.2	INTERVENTIONS	16
3.2.1	VILLAGE-LEVEL MASS TEST AND TREAT WITH HS-RDTs (MTAT-HS)	17
3.2.2	PEER NAVIGATOR-LED FTAT WITH HS-RDTs	20
3.2.3	ADHERENCE AND SAFETY MONITORING	22
3.3	STUDY EVALUATION METHODS	23
3.3.1	OVERVIEW OF EVALUATION ACTIVITIES BY STUDY OBJECTIVES, OUTCOMES, AND DATA COLLECTION ACTIVITIES	23
3.3.2	ACTIVITY 1 - EVALUATION OF TEST AND TREAT USING HS-RDTs	25
3.3.3	ACTIVITY 2 - ASSESSMENT OF HEALTH-SECTOR AND COMMUNITY FEASIBILITY AND ACCEPTABILITY OF THE TEST AND TREAT INTERVENTIONS	35
3.3.4	ACTIVITY 3 - ESTIMATING POPULATION SIZE OF FOREST-BASED HRPs	37
3.3.5	ACTIVITY 4 - ASSESS DEMOGRAPHIC AND GEOGRAPHIC PREDICTORS FOR G6PDD TESTING ADHERENCE WITH A POSITIVE <i>P. VIVAX</i> RDT	39
4	ETHICAL ISSUES RELATED TO HUMAN SUBJECT RESEARCH	39
4.1	ADEQUACY OF PROTECTION AGAINST RISKS	39
4.2	PROTECTION AGAINST RISKS ASSOCIATED WITH THE ADMINISTRATION OF AL / SLD-PQ	40
4.3	POTENTIAL BENEFITS OF THE PROPOSED RESEARCH TO THE PARTICIPANTS AND OTHERS	41
4.4	ALTERNATIVES TO PARTICIPATION	41
4.5	DATA AND SAFETY MONITORING PLAN	41
4.6	COLLECTION OF SPECIMENS	42
4.6.1	RACIAL AND ETHNIC ORIGIN	42
4.6.2	INCLUSION OF VULNERABLE SUBJECTS – CHILDREN AND PREGNANT WOMEN	42
4.7	SUBJECT CONSENT/ASSENT	43
4.7.1	PROCESS OF CONSENT	43
4.7.2	SUBJECT CAPACITY	43
4.7.3	SUBJECT/REPRESENTATIVE COMPREHENSION	43
4.7.4	DOCUMENTATION OF CONSENT	43
4.7.5	COSTS TO THE SUBJECT	43
4.7.6	PAYMENT FOR PARTICIPATION	43
4.7.7	FIELD DATA COLLECTOR TRAINING	43

4.7.8	COMMUNITY SENSITIZATION AND COMMUNICATION	44
4.7.9	STUDY ORGANIZATION AND MANAGEMENT	44
5	POTENTIAL RISKS, LIMITATIONS, DATA QUALITY ASSURANCE, AND DISSEMINATION PLAN	44
5.1	POTENTIAL RISKS FROM PARTICIPATION	44
5.2	LIMITATIONS	44
5.3	DATA QUALITY ASSURANCE PLAN	45
5.4	DISSEMINATION PLAN	45
6	BUDGET	46
7	TIMELINE	46
8	REFERENCES	48
9	APPENDICES	53
9.1	ANNEX A. DETAILED SCHEMATIC OF STUDY DESIGN	53
9.2	ANNEX B. STUDY POWER CURVES	54
9.3	ANNEX C. CROSS-SECTIONAL SURVEY INFORMED CONSENT FORM	56
9.4	ANNEX D. BASELINE/END-LINE HOUSEHOLD SURVEY QUESTIONNAIRE	56
9.5	ANNEX E. INDIVIDUAL BLOOD COLLECTION INFORMED CONSENT (CROSS-SECTIONAL SURVEY)	56
9.6	ANNEX F. BLOOD COLLECTION RESULTS FORM (CROSS-SECTIONAL SURVEY)	56
9.7	ANNEX G. MTAT HOUSEHOLD SURVEY INFORMED CONSENT FORM	56
9.8	ANNEX H. MTAT HOUSEHOLD SURVEY QUESTIONNAIRE	56
9.9	ANNEX I. MTAT BLOOD COLLECTION INFORMED CONSENT FORM	56
9.10	ANNEX J. FTAT SURVEY AND BLOOD COLLECTION INFORMED CONSENT FORM	56
9.11	ANNEX K. FTAT HRP SURVEY QUESTIONNAIRE	56
9.12	ANNEX L. G6PD TESTING REFERRAL LETTER	56
9.13	ANNEX M. G6PD HEALTH FACILITY REGISTER FORM	56
9.14	ANNEX N. ADVERSE EVENT REPORTING FORM	56
9.15	ANNEX O. SEVERE ADVERSE EVENT INVESTIGATION FORM	56
9.16	ANNEX P. FDG/KII INFORMED CONSENT FORM	56
9.17	ANNEX Q. MTAT TEAM FGD GUIDE	56
9.18	ANNEX R. PEER NAVIGATOR FGD GUIDE	56
9.19	ANNEX S. STUDY TEAM AND DISTRICT KII GUIDE	56
9.20	ANNEX T. TREATMENT ADHERENCE FOLLOW-UP FORM	56
9.21	ANNEX U. PEER NAVIGATOR FTAT COMPETENCY CHECKLIST	56
9.22	ANNEX V. PEER NAVIGATOR INTERVIEW GUIDE	56
9.23	ANNEX W. HRP INTERVIEW GUIDE	56

Acronyms

ACT	Artemisinin-based combination therapy
AE	Adverse event
AL	Artemether lumefantrine
CRCT	Cluster randomized control trial
CMPE	Center for Malariology, Parasitology, Entomology (Lao PDR)
DAMNs	District anti-malaria nucleus
DBS	Dried blood spot
DHO	District health office
DSMB	Data safety monitoring board
FSAT	Focal screen and treat
FGD	Focus group discussion
G6PDd	Glucose-6-phosphate dehydrogenase deficiency
GMS	Greater Mekong Subregion
HC	Health center
HCCA	Health center catchment area
HPA	Health Poverty Action
HRPs	High-risk populations (for malaria infection)
HRP 2/3	(<i>Plasmodium falciparum</i>) Histidine-rich protein 2/3
HS-RDT	Highly sensitive malaria rapid diagnostic test
HH	Household
IOM	International Organization for Migration
IRS	Indoor residual spraying
KII	Key informant interview
Lao PDR	Lao People's Democratic Republic
LLIN	Long-lasting insecticidal nets
MSAT	Mass screen and treat
MTAT	Mass test and treat
MMPs	Mobile and migrant populations
MSP	Merozoite surface protein
NIOPH	National Institute of Public Health (Lao PDR)
PAMS	Provincial anti-malaria station
PN	Peer navigator
PQ	Primaquine
RDT	Rapid diagnostic test
PCR	Polymerase chain reaction
SAE	Serious adverse event
SLD-PQ	Single low-dose primaquine
VMW/VMV	Village malaria worker/volunteer
VHV	Village health volunteer
WHO	World Health Organization

1 Protocol summary

Aim	<p>To determine the effectiveness, cost-effectiveness, acceptability, and feasibility of proactive targeted test and treat activities using high-sensitivity malaria rapid diagnostic tests (HS-RDTs) for reducing <i>Plasmodium falciparum</i> transmission among:</p> <p>(1) village residents in Champasak Province, Southern Lao PDR, and</p> <p>(2) mobile and migrant populations (MMPs) and other high-risk populations (HRPs) in Champasak Province, Southern Lao PDR.</p>
Primary research questions and hypotheses	<p><u>Null hypothesis 1</u>: There is no benefit of three rounds of village-based test and treat using HS-RDTs relative to standard of care for reducing <i>P. falciparum</i> parasite prevalence and health facility confirmed case incidence over a 12-month period.</p> <p><u>Research hypothesis 1</u>: Three rounds of village-based test and treat using HS-RDTs will be significantly more effective than standard of care (case management) at reducing <i>P. falciparum</i> parasite prevalence and health facility confirmed case incidence over a 12-month period.</p> <p><u>Null hypothesis 2</u>: There is no benefit of peer-navigator targeted test and treat using HS-RDTs relative to standard of care for reducing <i>P. falciparum</i> parasite prevalence and health facility confirmed case incidence over a 12-month period.</p> <p><u>Research hypothesis 2</u>: Routine peer-navigator targeted test and treat using HS-RDTs will be significantly more effective than standard of care at reducing <i>P. falciparum</i> parasite prevalence and health facility confirmed case incidence over a 12-month period.</p> <p><u>Null hypothesis 3</u>: There is no benefit of three rounds of village-based test and treat using HS-RDTs and routine peer-navigator targeted test and treat using HS-RDTs relative to standard of care for reducing <i>P. falciparum</i> parasite prevalence and health facility confirmed case incidence over a 12-month period.</p> <p><u>Research hypothesis 3</u>: Three rounds of village-based test and treat combined with routine peer-navigator targeted test and treat using HS-RDTs will be significantly more effective than standard of care at reducing <i>P. falciparum</i> parasite prevalence and health facility confirmed case incidence over a 12-month period.</p>
Research objectives	<p>Primary Objective: To evaluate the effectiveness of targeted test and treat activities using HS-RDTs compared to control for reducing the health center catchment- and village-level prevalence and incidence of <i>P. falciparum</i> within four districts in Champasak Province, Southern Lao PDR.</p> <p>Secondary Objectives:</p> <ol style="list-style-type: none"> 1. To determine key risk factors among study populations for malaria infection and identify optimal screening and case detection strategies addressing characteristics or behaviors that lead to increased risk

	<ol style="list-style-type: none"> 2. To determine the cost-effectiveness, acceptability, and feasibility of active case detection strategies aimed at reducing <i>P. falciparum</i> transmission among study populations, including peer navigator targeted test and treat among high-risk populations and village-based test and treat using HS-RDTs 3. To assess the sensitivity and specificity of HS-RDTs for the detection of <i>P. falciparum</i> parasites relative to PCR (baseline and end-line surveys, and peer-navigator activities), and compared to standard RDTs (baseline and end-line surveys and test and treat activities) 4. To assess the operational feasibility of malaria HS-RDTs for active case detection within the public health sector in Lao PDR 5. To estimate the size of the MMP/high-risk population in study districts in Champasak Province, Southern Lao PDR 6. To determine the effectiveness of test and treat activities in reducing <i>P. vivax</i> prevalence and incidence 7. To determine factors associated with G6PDd testing adherence after referral to district-level facilities for persons with <i>P. vivax</i> positive RDT results.
Study site and populations	<p>Total population of approximately 85,000 in 14 health center catchment areas.</p> <p>Health center catchments and forest-fringe population of four target districts in Champasak Province, Southern Lao PDR: Mounlapamook, Panthampone, Sanamsaboun, and Soukhuma.</p>
Study design	Split-plot community-randomized controlled trial, with peer navigator test and treat randomized at the health center catchment level, and village-based test and treat randomized at the village level within health center catchment areas.
Primary outcome measures	<ol style="list-style-type: none"> 1. PCR-based <i>P. falciparum</i> parasite prevalence in sampled villages 2. HS-RDT-based test positivity rate in village-level and HRP populations 3. Village-based population coverage of test and treat interventions 4. Village-based confirmed case incidence in sampled villages
Data collection and sampling period	12 months from start date (planned November 2017 – October 2018)
Sample sizes	<ol style="list-style-type: none"> 1) Household surveys: 5,600 persons aged > 18 months at baseline; 8,400 persons aged >18 months at end-line (total 14,000 persons during household surveys) 2) Village-based test and treat: approximately 22,000 persons per round 3) High-risk population test and treat: approximately 2,000 persons over 10-month period 4) Confirmed case incidence across 14 health center catchment areas: population of approximately 85,000
Statistical and analytical plan	Rapid reporting of RDT and HS-RDT positivity will be analyzed as interventions are implemented (or shortly thereafter) to provide preliminary assessment of interventions. Generalized linear mixed effects models will be used to test the

	effect of each intervention independently (main effects) relative to standard of care and additionally the combined interventions relative to standard of care (interaction term).
--	--

2 Background and introduction

2.1 Literature review

2.1.1 Active surveillance for malaria elimination

Malaria control programs traditionally use passive surveillance systems, which are based on symptomatic cases presenting to health facilities or community-based workers, to monitor disease burden. In elimination settings, where symptomatic case numbers are much lower, the World Health Organization (WHO) recommends incorporating active surveillance methods such as case investigation followed by active case detection in order to identify asymptomatic cases that may cluster around index cases in space and time.

Active case detection approaches are differentiated based upon whether they are conducted in response to an index case (reactive) or at regular intervals in locations known to have transmission, but not in response to a specific individual case (proactive). Reactive case detection (RACD) is commonly practiced by malaria elimination programs, and involves screening households in close geographic proximity to passively-detected index cases. Proactive case detection approaches can be conducted on community-wide scales (mass screen and treat (MSAT) or mass test and treat (MTAT)) or in small geographic areas (focal screen and treat (FSAT) or focal test and treat (FTAT)).

When employing standard RDTs, however, none of these strategies have been shown to have a sustained significant impact on malaria transmission due to limited sensitivity being insufficient to detect many persons with asymptomatic parasitemia [1–3]. In the Greater Mekong Subregion (GMS) specifically, few studies have been undertaken to assess the impact of active case detection, and in line with global findings, all have failed to demonstrate impact on community-level prevalence using standard RDTs [4,5]. While PCR-based strategies have shown potential impacts in the region [6], this methodology is not field deployable, and follow-up of cases may be difficult due to delays in reporting of results. A number of studies are currently exploring targeted mass drug administration (MDA) approaches, but due to concerns about large-scale MDA approaches in the GMS resulting from widespread artemisinin resistance, identifying optimal screen or test and treat activities is critical for accelerating progress to elimination in the region.

2.1.2 Active surveillance for malaria elimination in high-risk populations

In many pre-elimination and elimination settings, there is increasing evidence that the epidemiology of malaria is rapidly changing, with a greater proportion of transmission occurring outside of formal households, and in younger adult males [7]. This is especially true in the GMS including Lao People's Democratic Republic (Lao PDR), where forest-based activities are a major contributor to the overall economy in many areas. Forest-goers may have higher risk of malaria infection, and are likely linked by specific characteristics that increase their exposure to outdoor-biting and/or forest-dwelling mosquitoes. These shared characteristics may be social (people who socialize together), behavioral (people who sleep outside at night), related to travel (groups of people who go into the forest together) or occupational (people who work in or near the forest, on plantations, or at industry/development project sites). Despite a growing consensus that high-risk groups are often strongly linked by behavioral characteristics [8,9],

little evidence exists to help guide decision-making around which case detection strategies are most effective and how best to implement these strategies to target high-risk populations.

In the GMS, mobile and migrant populations (MMPs) are considered at highest risk for malaria due to their occupational environments (forest, forest fringe, plantations, and others) and commonly a lack of access or motivation to seek formal health care. While the tendency exists to refer to MMPs as a single homogenous group, the composition, exposures, and mobility patterns – and thus risk profiles, of these populations are thought to be highly heterogeneous.

Additionally, the proportion of reported malaria cases among males aged five years and older increased from 46 percent in 2009 to 86 percent in 2014, suggesting an association between malaria infections and occupational exposures, particularly among forest workers and migrant laborers [10]. Despite a growing emphasis on targeting MMPs, specific regional data on these subpopulations particularly in Lao PDR are limited. Furthermore, the relative contribution to transmission from forest-based work, compared to village-based risk factors, remains unclear.

MMPs and other forest-goers (hereafter, ‘high-risk populations’ or HRP) constitute a barrier to malaria elimination and artemisinin-resistance control efforts in the GMS and beyond. As a result of these groups’ movement patterns, HRP have the potential to introduce malaria parasites and perpetuate the spread of artemisinin resistance to new areas. Moreover, foreign MMPs pose a further challenge to national elimination efforts, as these populations may be less likely to seek formal care, and are therefore less likely to be captured through national reporting systems. Finally, the isolated, ambiguous, and overlapping composition of MMP subgroups defies simple classification for targeting and tailoring of interventions. To date there have been limited studies actively targeting highest-risk populations; one successful study targeted a set of border crossings in Southern Lao PDR and found high prevalence of parasitemia [11], but there has been limited success with social network-based approaches such as respondent-driven sampling in HRP [12,13].

The Lao Ministry of Health defines MMPs as any workers or their family members who migrate for economic or labor-related reasons within the country or across national borders [10]. The majority of MMPs are thought to be comprised of adult men and some women engaged in logging, agriculture, forest foraging activities, and industrial/development projects [9,14]. A recent Lao government decree (enacted in May 2016) implemented stringent regulations on all logging, resulting in rapid shifts in the livelihood activities of local populations. However, many other demographics aside from loggers spend time overnight in forest areas or forest-fringe farm sites, and may be contributing to ongoing malaria transmission. A formative assessment carried out by UCSF in these populations in 2016 found that small-scale spatial movements by local rice farmers who generally migrate from their home villages seasonally may be an important contributor to overall transmission (UCSF, 2016, unpublished report).

2.1.3 Rationale

While there has been limited impact of MSAT/FSAT with standard RDTs, major investments in improved RDTs have led to the next generation of histidine-rich protein 2 (HRP-2) RDTs for *P. falciparum*, with an expected ~10-fold greater sensitivity. The new highly sensitive Alere Malaria Ag P.f Ultra Sensitive RDT (<http://www.alere.com/en/home/product-details/alere-malaria-ag-pf.html>) (HS-RDT) has recently become commercially available, with a primary intended use being active case detection for individuals with low parasitemia. The increased sensitivity (approaching PCR-based testing) in a user-friendly and field-deployable diagnostic will address gaps in active surveillance and allow direct targeting of the asymptomatic reservoir, with the potential to dramatically change the malaria surveillance landscape in the GMS and beyond. This HS-RDT has not yet been tested in an active case detection setting.

The standard first-line treatment for confirmed uncomplicated *P. falciparum* malaria cases in Lao PDR is artemether-lumefantrine (AL); single low-dose primaquine (SLD-PQ) has recently been added to this regimen. While AL is effective at clearing the asexual form of the parasite, it does not clear the sexual form gametocytes, which are still produced for a short period following treatment with AL (median of 72 hours) [15], contributing to onward transmission. SLD-PQ has recently been shown to be safe and effective for rapidly clearing sexual stage parasites [16], and modeling work suggests a marginal benefit on transmission when added to AL in low transmission settings [17]. The clearance of *P. falciparum* gametocytes by addition of SLD-PQ to standard AL treatment has the potential to increase the impact of active case detection interventions in the GMS [18]; however this has yet to be tested in a controlled field setting.

Formative work by UCSF, Health Poverty Action (HPA), International Organization for Migration (IOM), and others in 2015-2017 suggests that many ‘forest-goers’ in Southern Lao PDR may be permanent residents of nearby villages, who may spend only a limited interval (2-5 days) in forest or forest fringe areas before returning to their home villages. While being village-based provides a probable entry point for health services, many infections may be asymptomatic and therefore unlikely to seek care through village malaria workers (VMWs) or with health centers. This formative work also suggests that village-based populations in Southern Lao PDR are highly accepting of household-based active case detection, and that utilizing these new HS-RDTs in this setting is unlikely to be met with opposition.

However, active outreach in other high-risk subpopulations by public sector health staff is challenging due to the illegal/semi-legal nature of some activities in forested areas, as well as socio-cultural barriers that may exist if Lao is not the primary language of target groups. Additional formative work suggests there are currently few congregation sites suitable for venue-based surveillance within the target areas, and moreover, worksites are few, small in scale, and temporary. Together these circumstances suggest that novel methods will be required to access HRPs for testing with HS-RDTs.

One promising technique to address similar challenges in sampling hard-to-reach groups that has been widely used in other disease areas (especially HIV/AIDS) is that of ‘peer-navigators’ (PNs) who are simply peer-group members who are directly supported to seek out any persons like themselves for testing and treatment, and to provide linkages to health services [19,20]. These positions have several important differences from other outreach methods. Specifically, PNs are full-time paid positions, who need to have shared socioeconomic level, racial/ethnic identity, common language(s), and should have shared “lived experience” as target groups [20]. Finally, their sole responsibility is active case finding and direct community outreach in target subpopulations.

HPA’s studies in 2015 found that some HRPs (true mobile and migrant groups) may also travel with their extended families to forest-based worksites. These family members generally stayed in temporary dwellings within forests, but some may also transit from forested areas to nearby villages and visit small shops for supplies [21]. However, the schedules and frequency of these visits is unknown, and the total number of such HRPs has not been estimated. Finally, the ratio of these forest-based HRPs to village-based groups is currently unknown, but a deeper understanding would allow for prioritizing interventions towards the Lao National Strategic Plan 2016-2020 goals.

In combination with novel strategies for accessing HRPs, we hypothesize that active case detection using the next generation of HRP-2 RDTs can help bridge gaps in identification of high-risk asymptomatic

individuals with low density parasitemia, allowing for targeting of this reservoir and thereby reducing transmission.

2.2 Significance

In direct support of the Lao National Strategic Plan, robust comparisons of the effectiveness and cost-effectiveness of targeted parasite detection strategies are needed to prioritize inherently limited resources. Towards these goals, this study will assess the role of HS-RDTs in active case detection at both the village-level and in forest-based HRP for decreasing prevalence and incidence of *P. falciparum* in target areas. The effectiveness of these interventions will be compared independently and in combination against areas with no study interventions (standard of care case management). The findings of this study will provide the Center for Malariology, Parasitology, and Entomology (CMPE) and partners with practical and direct guidance on the effectiveness of active case detection for village and forest-based HRPs with HS-RDTs and artemether lumefantrine plus primaquine (AL-PQ) to further shrink the parasite reservoir in Southern Lao PDR towards the 2030 elimination goals within the context of current malaria program interventions.

2.3 Primary aim

In Southern Lao PDR a range of partners are supporting CMPE to reduce the parasite reservoir through scale-up of ITNs and support for prompt care-seeking behaviors, which have had major impacts on incidence within the last 2-3 years. This protocol plans to evaluate a novel platform for targeted *P. falciparum* parasite detection in the highest risk populations, many of whom may become infected due to forest-based work. A community-randomized controlled trial will be used to assess the impact of active case detection using HS-RDTs in village-based populations through test and treat campaigns, and in forest-based HRPs through routine active case detection by peer navigators operating in selected health center catchment areas. Specifically, the primary aim of this study is to quantify the impact of village-based and peer navigator active case detection with HS-RDTs in HRPs against the standard of care using prevalence (primary outcome) and incidence (secondary outcome) of *P. falciparum* in selected health center catchment areas and villages.

2.4 Primary research questions and hypotheses

Within the context of CMPE's standard interventions in Southern Lao PDR, this project will specifically aim to answer the following primary evaluation research questions and hypotheses:

In target areas, are three rounds of test and treat using HS-RDTs in villages and routine test and treat using HS-RDTs in HRP sites more effective than no test and treat (standard of care) in reducing *P. falciparum* incidence and prevalence over a 12-month intervention period?

Null hypothesis 1: There is no benefit of three rounds of village-based test and treat using HS-RDTs over standard of care for reducing *P. falciparum* parasite prevalence and health facility confirmed case incidence over a 12-month period.

Research hypothesis 1: Three rounds of village-based test and treat using HS-RDTs will be significantly more effective than standard of care at reducing *P. falciparum* parasite prevalence and health facility confirmed case incidence over a 12-month period.

Null hypothesis 2: There is no benefit of peer-navigator targeted test and treat using HS-RDTs over standard of care for reducing *P. falciparum* parasite prevalence and health facility confirmed case incidence over a 12-month period.

Research hypothesis 2: Three rounds of peer-navigator targeted test and treat using HS-RDTs will be significantly more effective than standard of care at reducing *P. falciparum* parasite prevalence and health facility confirmed case incidence over a 12-month period.

Null hypothesis 3: There is no benefit of three rounds of village-based test and treat using HS-RDTs and peer-navigator targeted test and treat using HS-RDTs relative to standard of care for reducing *P. falciparum* parasite prevalence and health facility confirmed case incidence over a 12-month period.

Research hypothesis 3: Three rounds of village-based test and treat combined with peer-navigator targeted test and treat using HS-RDTs will be significantly more effective than standard of care at reducing *P. falciparum* parasite prevalence and health facility confirmed case incidence over a 12-month period.

2.5 Study overview

To test these hypotheses, this study will employ a split-plot community-randomized trial design for the two main interventions: MTAT using HS-RDTs in village-based populations, and PN-led FTAT using HS-RDTs in forest-based HRPs. The primary outcomes include: PCR-based *P. falciparum* prevalence at end-line; HS-RDT test positivity rate in village and HRP populations; and village-based population coverage of test and treat interventions. The trial will be implemented between November 2017 and October 2018 in Champasak Province, Lao PDR. The interventions will consist of three rounds of MTAT spaced throughout the year, and continuous FTAT by PNs in forest and forest-fringe HRPs. The primary evaluation will be conducted through an end-line cross-sectional survey in October 2018. A baseline cross-sectional survey will be conducted in November 2017 to gather baseline information and assist with study randomization.

2.6 Summary of ethical issues

All project activities will be approved prior to implementation by the institutional review boards in Lao PDR (NIOPH/MOH) and at UCSF.

Artemether-lumefantrine (AL) is the standard first-line drug for uncomplicated *P. falciparum* malaria in Lao PDR, and will be used to treat all positive cases detected during study interventions. Single-low dose primaquine (SLD-PQ) has recently been approved for use for uncomplicated *P. falciparum* malaria infection and in addition to AL constitutes the first-line drug regimen of policy.

The use of SLD-PQ for *P. falciparum* has been shown to have no clinically significant impacts on hematological indexes in populations with severe glucose-6-phosphate dehydrogenase deficiencies (G6PDd) in the GMS [22]. SLD-PQ is currently recommended by WHO without prior G6PD testing, and no serious adverse events (SAEs) are expected from SLD-PQ.

In all settings (village and forest-based), individuals with malaria infections identified by RDTs/HS-RDTs will benefit directly from treatment with AL-PQ. Furthermore, non-infected individuals within households and communities will benefit from population-level clearance of parasites including gametocytes. While the HS-RDTs specifically target the low-density infection *P. falciparum* reservoir, standard RDTs will likely identify *P. vivax* infections. Individuals with a confirmed *P. vivax* infection will benefit from treatment with AL (standard treatment) and will be referred to the nearest district hospital for G6PD testing and follow-up treatment with primaquine. It is also possible that some individuals with a low-density *P. falciparum* infection also have a low-density *P. vivax* infection. These individuals and their communities may benefit from clearance of blood-stage *P. vivax* parasites.

Household members and parents/guardians of children will be verbally informed of the general purpose of the test and treat interventions, the study, and the possible risks and potential benefits associated with participation in the research and intervention. All informed consent procedures will be conducted in the appropriate local languages. Participation in either the research or the intervention is voluntary. For children <6 years old, malaria testing will be based on consent from the parent or guardian; for children aged 6 to <18 years of age the child's assent will also be required.

The monitoring and evaluation of the interventions includes: a short survey at the time of the intervention documenting insecticide-treated mosquito net possession and use, any history of fever in the past two weeks, and any history of forest- or rice field-related work in the past month; routinely collected malaria incidence data from health facilities; cross-sectional household surveys with finger sticks for malaria parasitemia; and qualitative data collection with forest goers (open-ended questions) during implementation, and study team members (MTAT teams, PNs) and district health staff (focus group discussions and key informant interviews) at end-line. Participation in any of the research is voluntary, and will not affect an individual's ability to participate in the intervention. There is minimal risk from the evaluation measures.

3 Methodology

3.1 Study site

After years of declines, Southern Lao PDR experienced a resurgence of malaria incidence in 2013-2015, and incidence rates in Champasak Province remain among the highest in the country. The study will be carried out in selected health center catchments areas (HCCA) in four districts in Champasak Province (Mounlapamook, Panthampone, Sanamsaboun, and Soukhuma), targeting the highest-burden HCCAs ([Figure 1](#)) which include those that were part of the previous formative studies by UCSF in 2016. The study area population, number of villages, total health facilities, and *P. falciparum* and *P. vivax* annual parasite indices (API) for 2015 by district can be found in [Table 1](#).

Figure 1. Overview of project districts, Champasak Province, Lao PDR, showing all-species API (2015)

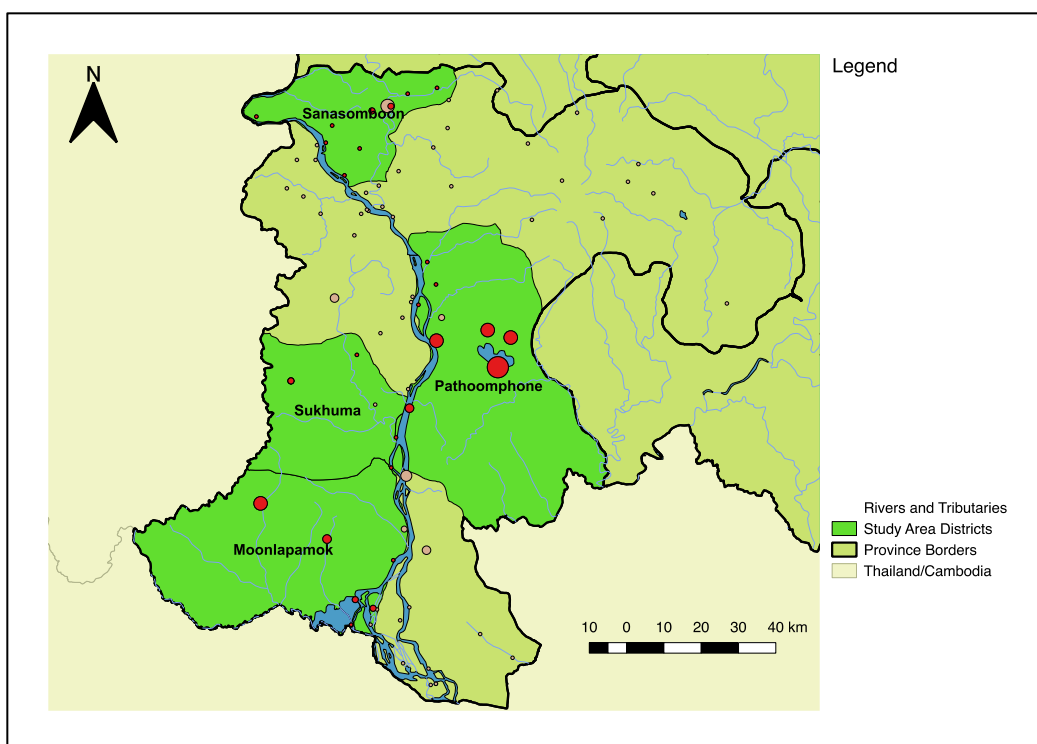


Table 1. Study area population and confirmed case incidence, Champasak Province, Lao PDR

District	Total villages	Total population (2015 census)	Total health facilities	<i>Pf</i> cases 2015 (API)	<i>Pv</i> cases 2015 (API)	Total cases 2015 (API)
Mounlapamook	50	38,800	8	944 (24.3)	1558 (40.2)	2593 (66.8)
Panthamphone	93	61,252	9	2529 (41.3)	2381 (38.9)	5082 (83.0)
Sanamsaboun	86	69,338	10	268 (3.9)	1564 (22.6)	1856 (26.8)
Soukhuma	62	58,445	5	639 (10.9)	788 (13.5)	1459 (25.0)

Currently, CMPE's standard-of-care interventions include periodic mass distribution of ITNs, health facility level testing with RDTs, community case management by VMWs in high-burden areas, and IEC/BCC work through village malaria/health workers. In some high-burden areas, malaria posts and mobile malaria teams have been implemented on a pilot-scale, but maintaining momentum in light of other competing priorities within the health sector has been challenging. There are currently no indoor residual spraying (IRS) activities, and case investigation and follow-up occur only in outbreak scenarios. The current first-line treatment for uncomplicated *P. falciparum* malaria is AL with SLD-PQ, however the use of SLD-PQ is a new policy that has not yet been widely implemented.

Multiple other partners are currently working in districts in Champasak. Clinton Health Access Initiative (CHAI) has been supporting supply chain management from national to district level through the mSupply system, and this is expected to expand to cover all districts in Champasak during the coming year. In addition, CHAI plans to place support staff at the provincial level in Southern Lao PDR to provide day-to-day support and technical assistance to strengthen the capacity of provincial and district staff, including in the four target districts in this study.

PSI recently launched a public-private mix (PPM) intervention project at selected private-sector facilities in five southern provinces in Lao PDR, including Champasak. This project provides financial, technical, and

managerial support to PPM network sites using a passive case detection strategy with real-time smartphone or tablet-based reporting of malaria cases. Scale-up is underway to potentially cover all clinics and pharmacies in this study's project districts.

A malaria post initiative funded by the Regional Artemisinin-resistance Initiative – Inter-country Component (RAI-ICC) and implemented by HPA was designed to pilot early diagnosis and treatment (EDAT) and enhanced surveillance in known malaria hotspots along the Lao PDR-Cambodia and Lao PDR-Thailand borders in Champasak. Included in this package of work is the establishment of malaria posts in hard-to-access forest areas (which otherwise lack access to formal healthcare); the distribution of forest survival kits to forest-goers and military personnel; EDAT training for malaria post volunteers (MPVs) and military nurses; and the provision of mobile phones to improve surveillance and commodity stock-out reporting. These forest kits include RDTs, AL drug treatments, mosquito repellent, flashlight/torch, paracetamol, bandages, gloves, and oral rehydration solution (ORS), among other supplies; however no mosquito nets are provided. While ICC ends in June 2017, continuation of these same activities has been requested in the latest Global Fund application and activities will resume, if approved, in early 2018. Only a subset of the 48 functioning malaria posts currently distribute the forest kits, but there are plans to expand this intervention in early 2018 to all 48 posts if the Global Fund approves the funding request.

HPA's other activities in Champasak include training, mobilization, and provision of incentives for VMWs; intensified active case detection in remote areas and development project sites; international border screening points; efforts to increase cross-border coordination; and malaria education campaigns in schools and through the Lao Women's Union.

Two qualitative studies have recently been completed in Southern Lao PDR to explore malaria-related knowledge and behavior in HRPs. A mixed-methods study was implemented in three provinces (Attapeu, Champasak, and Sekong) in Southern Lao PDR in 2015 [21] by HPA with assistance from UCSF and CHAI. A total of 189 HRPs were interviewed; of which two-thirds were of Lao origin, 31% were Vietnamese, with smaller numbers of Chinese, Cambodians, and Thais [21]. HRPs in these areas were found to be contactable, had diverse occupations and origins both within and outside of Lao PDR, and were highly welcoming of any health-based interventions due to reported limited access to services.

Subsequent formative studies through a partnership of UCSF, HPA, and CMPE in late 2016 expanded on these findings, and suggest a dynamic situation in Southern Lao PDR. Specifically, forest-going lifestyles have been impacted by depletion of hardwood and wildlife within forests due to widespread logging and hunting, as well as the recent government ban on logging. Villagers generally stated that free testing was a sufficient incentive for participation in test and treat activities, but health staff reported needing incentives to conduct active case detection activities. These studies also suggest that people with fever, whether coming from the forest, village, or field, know that malaria testing is readily available in both the government and private sectors. The findings from these assessments have been used to inform and fine-tune the sampling approaches, study population, eligibility criteria, logistical operations, and potential population entry points for this study.

3.2 Interventions

Previous work in Lao PDR suggests that the highest-risk populations generally have exposure to forest sites or forest-fringe farms, yet there are currently limited mechanisms within national guidelines to actively target these HRPs for screening and/or malaria testing.

The aim of this study is to assess the impact of two active case detection interventions using HS-RDTs: MTAT amongst village populations, and peer navigator-directed FTAT to target forest- and non-village based HRPs. Together these interventions aim to target the entire *P. falciparum* reservoir.

3.2.1 Village-level mass test and treat with HS-RDTs (MTAT-HS)

The village-level test and treat intervention will be implemented in three rounds during the period January 2018-October 2018. The three rounds will be conducted with at least one month separation between rounds, and not conducted during months with traditionally heavy rains, or during major crop harvesting periods, when large numbers of individuals may not be available during household visits. The preliminary timing for this three-round schedule is January, May, and August 2018.

Communities will be notified of the scheduled test and treat activities in advance to promote high levels of participation. In selected villages, all households will be visited by a MTAT team comprised of local district, health center, and village malaria staff, who will administer a short demographic and malaria risk factor survey to the head of household, and invite all household members aged >18 months to participate in RDT and HS-RDT testing. The survey responses will be used to assess whether any household members may be at higher risk for malaria, and may be developed in future MTAT rounds to serve as a screening form.

Testing strategy and operations: village-based MTAT-HS

At three time points during the year, MTAT teams comprised of local district, health center, and VMW staff will conduct test and treat activities in all intervention villages within a single calendar month. The sample frame will consist of all permanent households within villages; study teams will meet with village authorities before each intervention round to update all village-level household registers.

Teams will attempt to visit 100% of households in intervention villages during each round for testing and treatment of any positive cases. If based upon HS-RDT results and survey findings from the first two rounds, it is determined that the bulk of infected individuals have specific risk factors, a screening and treatment approach may be adopted whereby in the third round, individuals are initially screened and only those reporting specific risk factors are tested.

Individual consent will be obtained at each household and for each blood sample collected for testing. A finger stick blood sample will be collected for each consenting individual for testing with the HS-RDT, standard SD Bioline *Pf/Pv* RDT, and four blood spots on filter paper (baseline, end-line surveys and one MTAT round). Household members and parents/guardians of children will be verbally informed of the general purpose of the intervention and the study, and the possible risks and potential benefits associated with participation. All informed consent procedures will be conducted in the appropriate local language, and participants will be provided with a copy of the consent form with study contact information. Participation in either the surveys or the intervention rounds is voluntary. For children <6 years old, malaria testing will be based on consent from the parent or guardian. For children 6 to <18 years of age, the child's oral assent will also be required.

In the event that household members are not present at the time of testing, the MTAT team will ask if any absent household member has been ill with fever during the previous two weeks. If any absent household members are reported as having recent fever history or if any other household members test positive for malaria, the VMW will schedule a time to revisit the household. At this time, the VMW will administer the informed consent form to any previously absent household members and then test these individuals (and

treat positive cases). All individuals testing positive during the initial household visit will receive immediate treatment, as detailed below.

All individuals with suspected severe malaria or other severe illness as assessed by the MTAT team (including those with symptoms of severe anemia, prostration, impaired consciousness, respiratory distress, convulsions, circulatory collapse, abnormal bleeding, jaundice or passing dark urine) will be referred to the nearest health facility for clinical assessment and treatment.

All individuals who have provided informed consent, test positive by either HS-RDT or standard RDT, are ≥ 18 months old, are not pregnant or breastfeeding (see below), and who do not have symptoms associated with severe malaria or another severe illness, will be offered an age-appropriate course of AL (age-specific blister packages) and SLD-PQ according to national treatment guidelines [23].

Table 4. National guidelines for AL treatment

Age group	Body weight (kg)	Dose given daily for 3 days
6 months – 5 years	5 to < 15	1 tablet 2x per day
6 – 11 years	15 to < 25	2 tablets 2x per day
12 – 14 years	25 to < 35	3 tablets 2x per day
15 years and over	35 and over	4 tablets 2x per day

Note: all tablets are standardized at 20 mg artemether + 120 mg lumefantrine in age-specific blister packs.

All women with a positive HS-RDT or RDT test who are unsure of their pregnancy status will be offered a human chorionic gonadotropin (hCG) rapid pregnancy test. Women meeting any of the following criteria will be assumed to be in the first trimester and referred to the closest health center for treatment according to the national treatment guidelines (Table 5) [23]:

- Any woman declaring that she is in her first trimester of pregnancy, or who requests to receive quinine instead of AL;
- Any woman reporting that she is pregnant (or established by a positive rapid pregnancy test) of less than 3 months;
- Any woman reporting that she is pregnant (or established by a positive rapid pregnancy test) and reports no quickening.

Use of SLD-PQ is contraindicated for all women who may be pregnant or who are breastfeeding. Consequently, all women of reproductive age with a positive HS-RDT or RDT for *P. falciparum* will be offered an hCG rapid pregnancy test by study staff. All women with a positive hCG test or who choose not to be tested will not be given SLD-PQ, but treated with only AL as per national guidelines. It will be emphasized during all field team trainings that SLD-PQ can *only* be given to women of reproductive age with a confirmed negative hCG test.

Table 5. National treatment guidelines for pregnant women in Lao PDR

<i>P. falciparum</i> only	<i>P. vivax</i> only	Mixed <i>P. falciparum</i> / <i>P. vivax</i>
---------------------------	----------------------	--

First trimester	Quinine 30 kg/mg/day for 7 days	Chloroquine 25 mg/kg/day (divided in 3 days) - Day 1: 10 mg (base)/kg/day, once a day - Day 2: 10 mg (base)/kg/day, once a day - Day 3: 5 mg (base)/kg/day, once a day	Quinine 30 kg/mg/day for 7 days
Second or third trimester	AL for 3 days	AL for 3 days	AL for 3 days

Age and weight dosing regimens for national SLD-PQ treatment guidelines have not yet been finalized by CMPE/MOH, but are expected to follow standard WHO recommendations based on body weight [24]; age-based dosing has also been developed for use in Cambodia [25] but this has yet to be endorsed by the WHO. Measurement of lower doses is challenging due to the physical size of 15-mg tablets, and therefore SLD-PQ will not be administered to children weighing less than 10 kg. MTAT teams will be provided with scales to weigh all eligible participants in order to determine the correct SLD-PQ treatment dose.

Table 6. Single low-dose primaquine dosing guidelines

Body weight (kg) (WHO [24])	SLD-PQ dose (mg) (as base)	Age (years) (Ref [25])	SLD-PQ dose (mg) (as base)
< 10 kg	No treatment	0.5 – 4	2.5
10 to < 25	3.75	5-9	5
25 to < 50	7.5	10-14	7.5
50 to 100	15	≥ 15	15

A treatment guide based upon HS-RDT and RDT results for all individuals excluding pregnant and breastfeeding women and children <18 months is shown in Table 7.

Table 7. Treatment decision guide for RDT and HS-RDT results

RDT <i>Pf</i> / <i>Pv</i>	HS-RDT results	Action
(+ +)	(+)	Treatment aligned with national guidelines for mixed species infections: ACT (AL) with referral to district hospital for G6PDd testing
(+ -)	(+)	Treatment as per national guidelines for <i>P. falciparum</i> infections: ACT (AL) including SLD-PQ
(- -)	(+)	Treatment aligned with national guidelines for <i>P. falciparum</i> infections: ACT (AL) including SLD-PQ
(- +)	(+)	Treatment aligned with national guidelines for mixed species infections: ACT (AL) with referral to district hospital for G6PDd testing

(- +)	(-)	Treatment aligned with national guidelines for <i>P. vivax</i> infections: ACT (AL) with referral to district hospital for G6PDd testing
(+ +)	(-)	Treatment aligned with national guidelines for mixed species infections: ACT (AL) with referral to district hospital for G6PDd testing.
(+ -)	(-)	Treatment as per national guidelines for <i>P. falciparum</i> infections: ACT (AL) including SLD-PQ
(- -)	(-)	No treatment

3.2.2 Peer navigator-led FTAT with HS-RDTs

In Lao PDR, several types of health workers may also be forest-goers. Specifically, some VMWs/VHVs, and many malaria post volunteers, fulfill criteria for working as peer navigators (PNs), and have been trained by the national program to perform RDT-based testing. These health staff may be directly recruited or may be twinned with other forest-goers for the PN activities. Efforts will be made to recruit PNs fluent in Lao, Khmer, local languages, and potentially Vietnamese, wherever possible.

PN inclusion criteria include:

- At least 18 years of age
- Has resided in health center catchment area for at least the past six months
- Shared socioeconomic level and racial/ethnic identity with target HRPs
- Fluent in Lao and preferred proficiency in second local language (minority, Khmer, Vietnamese)
- Currently engaged in forest or field-based livelihood activities in target districts
- Literate and capable of administering informed consent
- Available for full-time employment as a PN and ability to travel via motorbike
- Highly familiar with health center catchment area geography and mobility/access routes
- Previous experience with malaria testing and treatment preferred, or interest and willingness to learn and undergo training by CMPE

The number of PNs recruited per HCCA will depend on the overall population size of the catchment, the malaria incidence, proximity to forest fringe and plantations including rice fields, and the estimated level of HRP activity in the area. On average, two teams each consisting of two PNs will be recruited for each intervention catchment area, totaling 28 PNs across the 7 intervention HCCAs. The 28 PNs will be allocated to HCCAs based on a combination of forest area and population to have roughly equivalent intervention effort per forest-going population in each HCCA.

PNs will be recruited for a 10-month period and provided with an initial comprehensive training followed by ongoing refresher courses throughout the implementation period. Training topics will cover proper informed consent procedures, data collection, ethical issues related to marginalized populations, the handling and use of RDTs, collection and storage of blood samples, provision of treatment to positive *P. falciparum* and *P. vivax* cases, referral of *P. vivax* cases for G6PD testing, and recognition of severe symptoms of illness or any adverse effects.

PNs will actively seek non-village based HRPs in forested areas, rice fields and plantations, and any other non-permanent settlements within target HCCAs, and conduct FTAT among all consenting individuals. By conducting continuous FTAT, IEC/BCC, and providing direct linkages between the target population and the formal public health sector, PNs aim to improve local HRPs' access to malaria testing, treatment, and information.

Specifically, PN responsibilities are expected to include the following (however tasks may be refined over time as opportunities and capacities are better understood):

- Identify potential HRPs in non-village sites in designated HCCAs on a continuous basis and obtain informed consent for the FTAT intervention
- As part of the FTAT intervention, administer a demographic and risk factor survey to the HRP participant to capture information on occupations and related exposures, travel history, intervention coverage, treatment-seeking behaviors, and acceptability and perceptions of FTAT
- Measure axillary temperature and perform standard RDT and HS-RDT on all consenting individuals; dried blood spots (DBS) will also be collected on all HRPs where feasible
- Administer treatment to any HRP testing positive by RDT or HS-RDT in accordance with guidelines outlined in [Table 7](#)
- Refer all *P. vivax* cases to the nearest district hospital for G6PDd testing and follow-up treatment
- Refer individuals with any severe symptoms or danger signs to nearest health facility for clinical assessment and treatment (PNs to undergo training on recognition of severe symptoms)
- Query all HRPs on estimated HRP population size ('wisdom of the crowds' method - whereby, in aggregate, individuals can estimate the total number of people like themselves)
- Provide identified HRPs with a study ID card for identification across arms and at health facilities
- At the consent of surveyed HRPs, geo-locate locations where FTAT activities take place
- Use a GPS logger to track where and when peer navigators conduct FTAT activities
- Invite a subset of forest-goer HRPs (especially those who are RDT-positive) to carry a GPS logger to track their spatial and temporal movement patterns; Facilitate retrieval of loggers through follow-up with participating HRPs
- Use IEC/BCC materials to provide malaria and general health information to all HRPs, and pinpoint nearest health facilities based on FTAT location for future care-seeking or treatment
- Provide personal contact information card with mobile phone number to all HRPs and encourage outreach if any questions or support for navigation/linkage to care is needed
- Invite HRPs (particularly those testing positive) to provide their contact information and that of their peers to allow for follow-up and exchange of information on potential sites for FTAT activities
- Work with HC workers to follow up on any of these social contacts who may be at higher risk for malaria infection
- Provide HRPs with a small token of appreciation for their participation (e.g., rechargeable torch)

Targeting strategy and operations: forest-based FTAT

PNs will target HRPs in forested and forest fringe areas, rice field regions, plantations, and in any informal settlements through three general mechanisms:

- i. Study health staff will first work with PNs in each HCCA to map out the boundaries of their assigned HCCA using specific natural boundaries and other landmarks to define the area within which they are working.
- ii. As piloted by HPA during formative studies, PNs will inquire about any potential HRP informal settlement locations or travel routes in study areas from all health staff, including HCs and village malaria posts. PN teams will then travel to these sites and assess any persons in these areas for inclusion, plus will gather any knowledge of other informal settlements or work sites in close proximity. Additionally, as optimal overnight sites in forest areas may be reused by

different forest-based groups, local knowledge will be used to target these areas for visits by PN teams.

- iii. Prior to project implementation, HC staff and VMWs will be retrained to ensure capture of relevant data from any non-village based persons presenting for malaria testing; while detailed locations or coordinates will not be possible, any geographic location should be captured.
- iv. On a biweekly basis during the study period, PN supervisors will contact HCs in target areas to review facility listings for any HRPs from potential non-village settings. Within health centers, all fever cases will be asked if they have spent any nights in the forest or rice fields in the past month. Cases who report recent forest or rice field travel will be asked to provide contact information for any persons they travelled or worked with.
- v. PN supervisors will arrange weekly mobile phone calls to support the PNs, address any urgent issues, and relay any up-to-date HRP information collected from HC staff. Every two weeks, the PNs will have a face-to-face meeting with their supervisors to rest, assess coverage and intensity of field activities to address any potential issues, map any forest sites, and plan the ensuing testing activities. This period will also allow for collection of any DBS samples collected and replenishment of field supplies. PNs will be incentivized through a monthly stipend, but per-capture payments or quotas will not be utilized to minimize potential biases in data collection. GPS loggers will be carried by PNs to track their movements, and periodically reviewed by field staff to ensure adherence to HCCA boundaries and comprehensive coverage of target area.
- vi. Throughout the study period, PNs will conduct forest-based testing and treating on a semi-continual basis, staying at field sites and forest encampments whenever possible.
- vii. If geographic overlap is evident between the two teams operating in the same HCCA, study staff will work with PNs to ensure comprehensive coverage of the HCCA area.
- viii. All HRPs contacted and enrolled will also be asked if they are willing to provide contact information for any other forest-goers related or known to them (especially those they have travelled with recently) for active follow-up by PNs.

At all of the sites visited, PNs will actively seek out any groups of persons like themselves (to be carefully defined during pilot testing) within defined and specific HCCAs, using GPS loggers to minimize spillover to adjacent areas (defined buffer zones will be delineated based upon HCCA mapping activities). The eligible study population will include all persons aged 15 years and older approached at any non-village sites within the defined HCCAs who spent at least one night outside a formal village in the past one month, including individuals traveling into the HCCA who are willing and sufficiently able to communicate with PNs to assess their eligibility. Treatment regimens will follow those under protocol section 3.2.1.

While the intensity and weekly schedules of FTAT activities will likely vary depending on seasonal patterns of crop harvesting and forest-going, PNs will be encouraged to continuously rotate throughout their designated HCCA in search of HRPs, drawing on information generated by HCs, VMWs, MTAT surveys, and previously-surveyed HRPs. The generation of unique personal identifiers using a combination of participant mobile phone numbers, age or date of birth, home village, and initials will allow for identification of any persons retested throughout the study period. When residents of villages within the HCCA are identified by PNs, PNs will identify the name of the home village and if possible obtain a GPS location for the individual's home.

3.2.3 Adherence and safety monitoring

Employing directly observed therapy on day 0 for both intervention arms will aim to maximize adherence with treatment. On day 0 of the household visits for the village MTAT intervention, and on day 0 for peer-navigator identified individuals, treatment with AL-PQ will be provided for all eligible individuals testing

positive by HS-RDT or RDT, and directly observed by the field team. Adherence to the full 3-day treatment course will be assessed for one round of village MTAT, whereby a list of all HS-RDT/RDT positive individuals administered treatment will be created and all individuals visited to assess completion of treatment and any side effects. Trained study staff will visit treated participants within 3-8 days of receiving the first treatment dose, and ask a series of questions to determine adherence with each dose, number of remaining tablets, and reasons for non-adherence or treatment refusal (Appendix T). Throughout the study period, any potential adverse events (AEs) will be referred to the nearest health center and documented as per 4.2 below.

3.3 Study evaluation methods

3.3.1 Overview of evaluation activities by study objectives, outcomes, and data collection activities

The study evaluation activities are linked to specific research objectives, outcomes, and data collection activities, as summarized in [Table 8](#) below.

Table 8: Overview of study outcomes and data collection activities

Study	Research objective	Primary outcome	Data collection activity
Activity 1: Impact evaluation of test and treat interventions using HS-RDTs	1. Evaluate the relative effectiveness of 3 rounds of MTAT-HS in village-based populations using AL-PQ, as compared to control, and the combination of MTAT-HS in villages and continuous FTAT in forest-based HRPs compared to control	<i>P. falciparum</i> prevalence in all persons aged ≥ 18 months	Cross-sectional parasite surveys (November 2017 and October 2018)
		Total and confirmed outpatient (OPD) <i>P. falciparum</i> malaria case incidence among all ages, matched to village by name	Routine malaria data from all reporting sites in districts (health centers, PPM sites, and district hospitals)
		HS-RDT and RDT test positivity rate	HS-RDT and RDT positivity data from village based MTAT-HS
	2. Evaluate the relative effectiveness of routine continuous FTAT in forest-based HRPs using AL-PQ, as compared to standard of care	Total and confirmed outpatient (OPD) <i>P. falciparum</i> malaria case incidence among all ages at the health facility level	Routine malaria data from reporting sites in districts (health centers, PPM sites, and district hospitals)
		HS-RDT and RDT test positivity rate	HS-RDT and RDT positivity data from FTAT in forest/rice-field based HRPs
	3. Assess and compare cost and cost-effectiveness of village-based and peer-navigator based case detection activities	Cost using standard procedures for costing methods and cost-effectiveness [27] and will be based on a health-sector (provider) perspective.	Program data on cost combined with estimates of program effectiveness

	4. Assess sensitivity and specificity of HS-RDTs relative to standard RDTs and to PCR-based parasite detection	<i>P. falciparum</i> prevalence by each testing methodology	Cross-sectional parasite surveys November 2017 and October 2018 (PCR, HS-RDTs and RDTs); village MTAT and forest-based FTAT surveys (HS-RDTs and RDTs)
	5. To determine the effectiveness of test and treat activities on reducing <i>P. vivax</i> prevalence and incidence	<i>P. vivax</i> prevalence in all persons aged ≥ 18 months	Cross-sectional parasite surveys (November 2017 and October 2018)
		Total and confirmed outpatient (OPD) <i>P. vivax</i> malaria case incidence among all ages, matched to village by name	Routine malaria data from all reporting sites in districts (health centers, PPM sites, and district hospitals)
Activity 2: Assessment of the feasibility and acceptability of MTAT and FTAT interventions as perceived by the health-sector, communities, HRPs, peer navigators, and study staff	1. Assess the acceptability, feasibility, support for, and participation in the MTAT/FTAT interventions	Proportion of survey respondents who strongly disagree, disagree, are ambivalent, agree and strongly agree on the importance and acceptability of community-based MTAT	Cross-sectional surveys and qualitative studies at end-line with health sector staff including VMWs and study staff
		Proportion of HRP survey respondents who strongly disagree, disagree, are ambivalent, agree and strongly agree on the importance and acceptability of peer navigator-based FTAT	Peer navigator directed FTAT surveys; interviews with participating HRPs
		Proportion of peer navigators who rate conducting the FTAT intervention as very easy, somewhat easy, somewhat difficult, and very difficult, and changes in proportions over time	Entrance, mid-FTAT, and exit interviews with peer navigators; focus group discussion with peer navigators at end-line
	2. Assess the capacity of peer navigators to conduct FTAT, and their confidence in ability to perform FTAT functions	Proportion of peer navigators receiving a 'pass' score on competency checklist during FTAT observation by supervisor, and change in proportion of 'pass' scores over time;	Supervisor observation of peer navigators conducting FTAT and scoring of competencies using checklist; FTAT survey data including peer navigator adherence to diagnosis, treatment, and referral guidelines
		Proportion of peer navigators rating their confidence in conducting the FTAT intervention as very low, low, moderate (confident), and high, and changes in proportions over time	Entrance, mid-FTAT, and exit interviews with peer navigators; focus group discussion with peer navigators at end-line
Activity 3: Estimate population size of forest-based HRPs	1. Estimate the total forest-based HRP population in targeted health center catchments	Total population size estimates triangulated through three separate estimations ('wisdom of the crowds', single-source capture-recapture, and double-list capture-recapture.)	Peer navigator directed FTAT surveys.

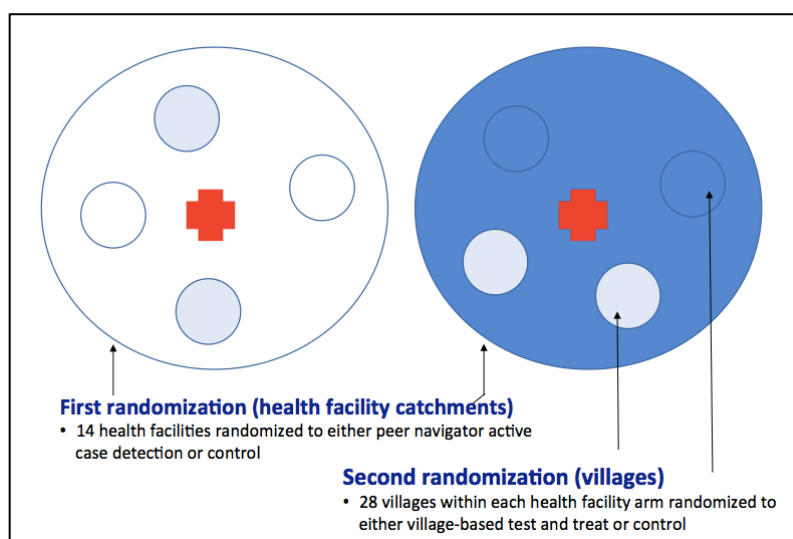
Activity 4: Explore demographic and geographic predictors for G6PDd testing referral adherence among positive <i>P. vivax</i> RDT cases	2. Determine risk factors for non-adherence for G6PDd testing at district hospital sites after referral	Proportion of symptomatic and asymptomatic referred cases presenting at district hospital for G6PDd testing.	Village-based MTAT surveys; peer navigator directed FTAT surveys; district hospital facility records.
---	---	--	---

3.3.2 Activity 1 - Evaluation of test and treat using HS-RDTs

3.3.2.1 Study design

To meet the overall aim and objectives, this study will employ a cluster-randomized control trial (CRCT) and randomize the interventions in two separate stages in a split-plot design (Figure 2 and Table 9).

Figure 2. Schematic of split-plot study design



A split-plot design [28–30] allows for randomization at two different scales, allowing one set of treatment/control (‘sub-plot’) areas to be nested within the second set of treatment/control areas (‘whole plot’). In this design, the first level, or ‘whole plot’ is often an intervention that is difficult operationally to conduct at a lower level. Randomization at two different spatial scales is necessary within this study, as PNs will be operating in areas between villages, so randomization at the village level will not be feasible. Conversely, the test and treat campaigns will be organized at the village level to maximize the number of units available for randomization to ensure adequate study power. The split-plot design allows for greater power to detect differences in sub-plot effect sizes as well as the interaction between sub-plot and whole plot.

3.3.2.1.1 Randomization

Randomization 1: Health center selection and randomization

A total of 14 HCCAs will be selected for inclusion based upon HCCA-level API and distance between HCCAs. HCCAs with higher API will be prioritized to improve power; where possible, directly neighboring HCCAs will not both be included to reduce contamination. Restricted randomization of the 14 HCCAs into either PN or control arms will be conducted, whereby HCCAs will first be matched into strata based upon HCCA-level API, population size, amount of forest cover, and non-contiguity of opposite arms. Within matched strata, the random allocation rule will be used to assign 7 HCCAs each to the PN or control group. Restricted randomization in this way will ensure balance across intervention and control groups on potential confounding factors. The 'sample' command in Stata v14 (StataCorp, College Station, TX) will be used to implement randomization.

Randomization 2: Village selection and randomization

All villages within selected HCCAs will be mapped and restricted randomization conducted for 28 villages from among each of the two HCCA arms from randomization 1 (PN FTAT or control). Randomization 2 will randomize the 56 total study villages to either MTAT or control (no MTAT intervention).

For each arm of randomization 1, suites of 28 villages that maximize distances between villages and/or include non-study buffer villages will be generated. From amongst these sets, a single set of 28 villages will be randomly selected for each arm of randomization 1.

A baseline survey will be performed approximately one month prior to implementation of interventions (described below). As baseline demographics and parasitemia are expected to vary widely across the study area, these survey data will be used to provide a well-balanced set of clusters. Restricted randomization of the 28 villages within each arm to either MTAT or control will be conducted, whereby villages will first be matched into strata based upon population size, distance to nearest forest, distance to health facility, parasite prevalence, intervention coverage, and other key covariates. The 'sample' command in Stata v14 (Stata Corp, College Station, TX) will be used to implement randomization.

Table 9: Split-plot community randomized controlled trial design

Study arm	Treatment group	Intervention description	Number of HCCAs	Number of villages/village clusters per arm
In health center catchments without peer navigators (total of 7 HCCAs)				
1A	Village-based test and treat	All members of target household population will be offered RDT-based testing with standard and HS-RDTs, with anyone testing positive for malaria with any RDT offered treatment with AL + SLD-PQ for clearing asexual and sexual stage parasites*	7	14
1B	Control	Standard of care		14
In health center catchments with peer navigators (total of 7 HCCAs)				
2A	Village-based test and treat	All members of target household population will be offered RDT-based testing with standard and HS-RDTs, with anyone testing positive for malaria with any RDT offered treatment with AL + SLD-PQ for clearing asexual and sexual stage parasites,* overlaid with peer navigator-based test and treat.	7	14

2B	Control	Standard of care, overlaid on peer navigator-based test and treat.		14
----	---------	--	--	----

* Excluding children <18 months and women who are pregnant, who believe they may be pregnant, or who are breastfeeding.

If possible, a public event will be held during which village elders randomize the units for full transparency of intervention allocation [31].

Allocation concealment

After allocation, the specified interventions will be implemented in HCCAs and villages, but assignments will not be blinded to participants or investigators due to the nature of the interventions. However, all efforts will be made to ensure data collection teams will be blinded to these allocations for the end-line cross-sectional survey. For analysis of all primary outcomes the study assignment arm will be blinded by use of concealed codes within the final dataset.

3.3.2.2 Primary outcome measures

1. Community-level PCR-based *P. falciparum* parasite prevalence in sampled villages: defined as the proportion of individuals >18 months old with *P. falciparum* infection (detected by PCR) out of all individuals >18 months tested within the 2017 and 2018 surveys.
2. HS-RDT-based test positivity rate in village-based and forest-based samples: defined as the proportion of all individuals tested by HS-RDT at each round of the MTAT interventions or during routine FTAT, with a positive HS-RDT, among the population older than 18 months.
3. Village-based population coverage of test and treat interventions: this indicator will be measured in two ways for each round of village MTAT. Operational program coverage is defined as the proportion of individuals ≥18 months old and households visited and offered the MTAT interventions within the target areas. Effective program coverage is defined as the proportion of individuals (≥18 months old) that agreed to participate in the MTAT intervention among all individuals ≥18 months old eligible to participate in the intervention in the target population.
4. Community-level confirmed *P. falciparum* malaria parasite incidence: defined as the number of OPD malaria confirmed and suspected cases per person per year for each village, as ascertained from the health facility registers, utilizing village population size estimates for the exposure denominator.

3.3.2.3 Sample size

The study design is constrained by the number of villages and HCCAs available for randomization. The maximum number of health center catchments available is roughly 28, as other health center catchments currently have multiple partners implementing. Within these 28 HCCAs, there are a total of roughly 230 villages (5-10 villages per HCCA). Within the 14 highest API HCCAs to be randomized at the first stage, there are 121 villages, of which 56 will be randomized.

The primary outcome will be based on comparing differences in parasite prevalence between intervention and control areas; while confirmed incidence will be a secondary outcome, it will not be used to determine sample sizes, as there are limitations with case diagnosis and reporting within the Lao public health sector.

3.3.2.3.1 Community-level malaria parasite prevalence

The number of village clusters and size needed for valid statistical comparisons was calculated using a simulation-based approach, as direct analytical methods have not been developed for a split-plot cluster-randomized trial [32]. Simulations were performed using R software [33] and the *sim.glm*-package [34]. Power calculations for varying numbers of health facilities, villages, and sample sizes per village are shown in Appendix I.

Sample size for the primary evaluation at the end-line survey was determined based on the power to detect a difference between MTAT and control, as well as between MTAT combined with PN FTAT and control, assuming a 30% reduction in prevalence in PN areas compared to control. With an assumption of 4% *P. falciparum* prevalence via standard PCR (or uPCR – to be determined following baseline survey) and a between facility and village variance of 0.10, in order to detect a 50% difference in the prevalence of *P. falciparum* between MTAT and control, with 80% power at a 5% significance level, a total of 14 village clusters per arm (for a total of 56 villages across all arms) with 150 persons sampled per cluster (30 HH, average HH size of 5) are required, amounting to a minimum total sample size of 8,400 persons, or 1,680 households for the end-line survey. To account for potential non-response rates of 10% at the HH-level, a total of 1,848 households, or 33 per village, will be sampled.

This total sample size will permit an independent comparison between the village-level test and treat intervention and control, as well as the comparison between the combined village and PN test and treat interventions and control. These power calculations assumed a realistic 30% reduction in prevalence based upon the PN intervention independently; while the trial will not have adequate power to detect this level of difference, a reduction of 50% is detectable.

Sample size for the baseline survey is based on the precision for comparisons of primary outcomes by arm at baseline. Using the 56 selected villages, a total of 20 households will be sampled per cluster (100 individuals, average HH size of 5), amounting to a minimum total sample size of 1,120 households and 5,600 persons for the baseline survey. To account for potential non-response rates of 10%, a total of 1,232 households and 6,160 persons will be sampled. With an assumed *P. falciparum* prevalence of 4.0%, and 3,080 sampled per arm, this sample size will provide precision of 4.0% (95% CI: 3.3-4.7%; binomial exact) in each arm.

3.3.2.3.2 HS-RDT test positivity during interventions

Test positivity by HS-RDT will be monitored for all rounds throughout the intervention period. There is no formal sample size calculation for this outcome, as all residents of selected villages will be enrolled. Per round, an estimated 22,000 individuals will be tested.

There are currently no estimates of the total number of non-village-based HRPs in Lao PDR. However, assuming that parasitemia in these high-risk individuals will be approximately 5% by any RDT (assuming consistent numbers with previous data from higher transmission periods of 11.5% by PCR; and 8% by standard RDT [11]), with a target to enroll a minimum of 2,000 forest-working HRPs over the course of the study, this will provide an estimate of 5% (95% CI: 4.1 – 6.0%; binomial exact) for parasite positivity by RDT over the course of the study. These values will help to quantify the relative burden of parasitemia in HRPs compared to the village populations for better targeting of interventions.

3.3.2.3.3 Community-level confirmed *P. falciparum* malaria parasite incidence

The confirmed parasite incidence from all reporting units (including HCs, district hospitals, VMWs with RDTs, and PPM sites) will be captured throughout the study with support from study staff. In the months

prior to the start of study activities, all villages will be mapped, and trainings conducted with health facility staff to systematize the collection of village names at health facilities for confirmed malaria cases. Routine supervision of health facility staff will be conducted to ensure accurate recording of village names in health facility registers throughout the trial.

Using simulation-based sample size calculations with Poisson-distributed annual case counts per village, an expected baseline *P. falciparum*-specific API of 6 per 1,000, with 14 HCCAs, a between village coefficient of variation of 0.004, a between facility variation of 0.003 (Lao stratification database, variance of API across all included HCCA = 0.0039), and an average village population size of 750 (750 person-years of follow-up), the study will be adequately powered at 0.90 to detect a 50% reduction in *P. falciparum* incidence for the test and treat intervention, and at 0.80 to detect a combined 50% reduction due to village-based test and treat, and a 30% reduction due to PN test and treat.

3.3.2.4 Data collection

3.3.2.4.1 Cross-sectional surveys

At the baseline and end-line, cross-sectional surveys will be conducted to obtain an unbiased estimate of *P. falciparum* malaria parasite prevalence in each study arm (November 2017 and October 2018). The baseline survey will be used to obtain baseline demographics, intervention coverage, and both standard and HS-RDT-based parasite prevalence to inform restricted randomization of study villages, and to assess the prevalence of any HRP2/3 deletions. The end-line survey will also test with both RDTs, but will be focused on the primary outcome for the overall study using PCR-based testing.

Sampling frame and sampling strategy

Within each of the 56 villages selected for study inclusion, survey staff will work with village authorities to update household ledgers, and then all households within each village will be enumerated and a GPS point captured. All households will be given a study ID card and household sticker with a unique barcode, which will be used throughout the study to identify repeat visits at each household, as well as for individuals to present at health facilities if they report for care.

In order to reduce potential contamination due to proximity to intervention areas (for the control arm), households within 2 km of a neighboring study village will be removed from the sampling frame. For each of the 56 villages, households will be selected via simple random sampling from the remaining HH lists.

Questionnaires and human specimen collection

During each survey, selected households will be visited and the head of household interviewed by a study staff member using a tablet or paper form. To capture any household members not present at time of survey, study staff will plan for an overnight stay whenever feasible to schedule visits for early morning or late evening, but will have a maximum of four visits to each HH. The survey questionnaire used for interviewing will be developed in English with input from local health staff. This will then be translated to Lao, and back-translated by a fluent bilingual health expert prior to field testing.

The survey questionnaire will capture household-level demographics, and assess potential risk factors for malaria infection. Information collected will include age, gender, pregnancy, nationality, ethnicity, occupation, socioeconomic status, travel history, history of malaria, treatment seeking for fever in the past two weeks, individual and household use of vector control measures, housing structure type, proximity to forest and forest-fringe, and frequency of overnight sleeping in forest or forest-fringe areas.

All household members (residents and temporary visitors) aged 18 months and older will be invited to participate in an RDT and blood collection component. Informed consent will be obtained from all participants, including parental consent for any participant younger than 18 years of age. After consenting, the study team will capture axillary temperature, and test each individual using both a standard RDT (CareStart Ag Pf/Pv, SD Bioline Cat #05FK80) and HS-RDT (SD Bioline Malaria Ag P.f High Sensitive Cat# 05FK140), followed by collection of four DBS on filter paper.

If found positive by RDT or HS-RDT, treatment will be administered as in [Table 7](#); women of reproductive age will be assessed for treatment as in section 3.2.1.

3.3.2.4.2 Test and treat data collection

The test and treat campaigns will consist of two independent intervention activities. The first will be three rounds of village-based test and treat (MTAT) of all selected villages, and the other activity will be peer-navigator directed test and treat (FTAT) in forest- and field-based populations captured in areas outside of formal settlements in selected HCCAs. Data collection during village-based MTAT campaign rounds and PN FTAT is described below.

Questionnaires and human specimen collection: village-based MTAT-HS

For all households in intervention villages, after obtaining informed consent, a short demographic and malaria risk factor survey will be conducted to obtain information on household members' occupations, recent forest work or travel, ITN usage, and recent care-seeking behavior for fever.

All household members (residents and temporary visitors) aged 18 months and older will be invited to participate in RDTs (all rounds) and blood collection component (one round only, as below). Informed consent will be obtained from all participants, including parental consent for any participant younger than 18 years of age. After consenting, the MTAT team will capture axillary temperature, and test each individual using both a standard RDT (CareStart Ag Pf/Pv, SD Bioline Cat #05FK80) and HS-RDT (SD Bioline Malaria Ag P.f High Sensitive Cat# 05FK140).

If found positive by RDT or HS-RDT, treatment will be administered as in [Table 7](#); women of reproductive age will be assessed as in section 3.2.1.

All patients with a *P. vivax* positive RDT (both febrile and asymptomatic) will be given a coded and signed informational letter directing them to the nearest district hospital for G6PDd testing and follow-up treatment.

For one of the MTAT rounds, DBS will also be collected from a randomized 25% subset of all households (approximately 6,800 samples) for subsequent PCR-testing to assess any factors related to overall impact of the intervention.

Questionnaires and human specimen collection: PN FTAT

For all individuals identified by PNs in intervention HCCAs, after obtaining informed consent, a short demographic and malaria risk factor survey will be conducted to obtain information on the respondent's occupations, recent forest work or travel, ITN usage and recent care-seeking behavior for fever.

All individuals will be invited to participate in RDT-based testing (standard RDT and HS-RDT) and a blood collection. Informed consent will be obtained from all participants. After consenting, the PNs will capture axillary temperature, and test each individual using both a standard RDT (CareStart Ag Pf/Pv, SD Bioline

Cat #05FK80) and HS-RDT (SD Bioline Alere Malaria Ag Pf High Sensitive Cat #05FK140), followed by collection of four DBS on filter paper.

If found positive by RDT or HS-RDT, treatment will be administered as in [Table 7](#), and women of reproductive age will be tested and treated as in section 3.2.1.

All patients with a *P. vivax* positive RDT (both febrile and asymptomatic) will be given a coded and signed informational letter directing them to the nearest district hospital for G6PDd testing and follow-up treatment.

PNs will provide participants with a small gift for participation (e.g., torch or topical repellent). Sites where interviews occur will be geo-located if all parties agree. PNs may also obtain contact information from consenting persons to allow for follow-up on positive cases and potential creation of support networks for other HRPs.

A subset of forest-goers (all RDT-positives and a subset of RDT-negatives) will be invited to carry global positioning system (GPS) loggers ([Figure 3](#)) to capture their movement patterns in time and space. As these units require data to be downloaded, study staff will facilitate subsequent retrieval of units from HRPs using small incentives (e.g., phone top-up cards), and will use these contact events to enroll other HRPs whenever possible.

Figure 3. “I-GotU” personal GPS data logger



3.3.2.4.3 Costing data collection

Itemized expenditure data will be collected during household test and treat and peer-navigator led activities ([Table 10](#)). This will allow calculation and comparison of the cost-per-case identified using both types of test and treat approaches.

Table 10. Types and potential sources of expenditures for per-case costings from focal test and treat activities

Expenditure category:	Types of expenditure data required	Potential information sources
Personnel	All human resource expenditures and time contributions, including: <ul style="list-style-type: none"> · Salary/wage payments · Volunteer labor/per diems 	<ul style="list-style-type: none"> · Annual program budgets for salaries and benefits · Work logs or weekly reports from health staff, VMWs, and PNs

Commodities and Services	<p>All supplies and services used toward test and treat activities:</p> <ul style="list-style-type: none"> · In-kind donations · Purchased commodities (e.g., acquisition costs, duty fees) · Travel/transit costs: fuel, transit fees and services, airfare · Reproduction costs, postage · Staff trainings: per diem payments, trainer fees, consultants · Warehouse space 	<ul style="list-style-type: none"> · Printing, shipment receipts · Training budgets and receipts · Vehicle travel logs, fuel receipts · Utility bills · Consultant invoices
---------------------------------	--	--

3.3.2.4.4 Laboratory procedures and analysis including PCR

The laboratory procedures described below will be followed for all laboratory-based activities conducted during this project. The laboratories performing these tests include the Pasteur Institutes in Phnom Penh and Paris, and UCSF.

RDTs will be performed in health facilities and during field activities using quality-assured lots. Dried blood spots (DBS) will be regularly transported to the district or provincial offices for refrigerated storage prior to bulk transport to Vientiane, and shipment to designated laboratories. DNA from DBS will be extracted using the Chelex method [35], and tested by RT-PCR with subsequent speciation for all samples found to be *Plasmodium*-positive.

Serology, a test of past infection as assessed by the presence of antimalarial antibodies, will be used to improve the identification of hotspots and estimate current and historical transmission intensities [36]. Using DBS, ELISA assays will be performed using previously described methods. Briefly, antibodies will be eluted from DBS and assayed to detect antibodies against the *P. falciparum* blood stage antigens including merozoite surface protein-1 (MSP-1) and apical membrane antigen-1 (AMA-1), both biomarkers of *P. falciparum* exposure [37]. Markers for *P. vivax* exposure will include Pvmsp-1 and Pvcsp [38]. Other antigens that are sensitive and specific for recent exposure (currently undergoing evaluation) for *P. falciparum* and *P. vivax* may also be used. ELISA assays will be performed in duplicate and optical densities recorded with an ELISA reader. Other serological and antigenic platforms (bead array, protein microarray) may be used to analyze responses to multiple antigens/antibodies, if available.

Genotyping of *P. falciparum* will consist of a panel of microsatellites located throughout the genome. Briefly, DNA samples will be amplified in a multiplex pre-amplification step followed by amplification of microsatellites in individual reactions using fluorescently tagged primers, and sized using denaturing capillary electrophoresis. Multilocus genotypes from mixed infections will be reconstructed, where possible, by quantifying alleles at each locus. Genotyping of additional loci including for HRP2 deletion will be performed as needed.

Individual microsatellite amplifications will be undertaken using single round or nested PCR assays with fluorescently labeled primers, and the amplicons sized by denaturing capillary electrophoresis with internal size standards. Allele-calling will be undertaken with the aid of the GeneMapper v4.0 software. The potential for effective multilocus haplotype reconstruction in polyclonal infections will be explored. Additional informative SNP markers identified in whole genome sequencing efforts may also be genotyped as necessary to improve sample fingerprinting.

Genotyping for markers of drug resistance will include PCR and/or sequencing to identify markers of resistance to artemisinin-based combination therapies (ACTs), in particular artemether-lumefantrine,

artesunate-amodiaquine, dihydroartemisinin-piperaquine (including K13), and the antifolate combination sulfadoxine-pyrimethamine (SP).

Human DNA may be used in the future to screen for genetic risk factors for malaria, such as inherited blood disorders, e.g., G6PD status. This information may be used to assess the prevalence of G6PD deficiency in study areas to support CMPE's national-scale implementation of primaquine for *P. vivax* (full-course) and *P. falciparum* (single low-dose).

3.3.2.4.5 Sample storage and transfer

DBS sample cards consisting of four individual spots of blood will be collected by finger prick from all persons screened in the baseline and end-line surveys, from 25% of households for one round of MTAT, and PN FTAT activities when feasible. These DBS cards will be left to air-dry before storage in Ziploc bags containing desiccant to limit build-up of moisture. DBS cards will be stored at 4°C within one week, and at -20°C within one month; extracted DNA will also be stored at -20°C. Samples will be kept securely in Vientiane and/or Phnom Penh and/or San Francisco for current and future studies. Transfer of samples outside of Lao PDR will be at the specific request of CMPE.

3.3.2.5 Analytic plan

Data will be collected via ODK-based tablet application with internal range checks or paper-based with subsequent double-entry, and will be stored in Microsoft Excel or Access. The number of malaria cases, based on RDT and PCR results, will be mapped by village or sub-village and compared between surveys.

Where possible, environmental covariates including forest cover will also be utilized; and geostatistical analyses will be conducted to produce risk maps for the study districts.

Primary outcomes

Outcome 1: Community-level PCR-based *P. falciparum* parasite prevalence in sampled villages

The effectiveness of the interventions will be assessed as *P. falciparum* prevalence via PCR at end-line (post only) using generalized linear mixed effects models with separate random intercepts to allow for clustering within villages and health center catchments. The binomial distribution will be used to analyze prevalence outcomes (logistic regression). All main analyses will be analyzed as intention-to-treat, and all survey clusters will be analyzed within the intervention group assigned at randomization, regardless of adherence. The primary effect estimate will be evaluated using the fixed effects for village MTAT intervention, PN FTAT intervention, and an interaction term for the combined interventions. Secondary analyses will include adjustment for age, sex, health center-catchment and village-level baseline prevalence of *P. falciparum* parasitemia by PCR, and other potential confounders, and a per-protocol analysis of the primary effect estimate.

Outcome 2: HS-RDT-based test positivity rate in village-based and forest-based samples

The HS-RDT and RDT test positivity rate will be estimated for the MTAT villages during each intervention round. This will be done as soon as data on HS-RDT results are available, which would be expected to be one month following each round. Differences in prevalence measures at each round will be assessed using a χ^2 test, as well as logistic regression models to account for potential confounding factors.

Outcome 3: Village-based population coverage of test and treat interventions

The operational coverage will be estimated at the individual and household level as the percent of the population that received a visit from the intervention teams to offer the MTAT interventions, among those

eligible for inclusion. This will be obtained from a combination of MTAT program data and enumeration data. Additionally, the proportion of individuals accepting the MTAT interventions ≥ 18 months, among those eligible for inclusion in the intervention, will be estimated, providing an estimate of the effect coverage of each program. Data for the denominator of individuals and households targeted for the intervention will be ascertained from the household enumeration for the sampling frame. Additionally, to validate the enumeration, attempts will be made to use remote sensing data, and/or Google Earth, to enumerate household structures. To the extent possible, individual, household and community level factors associated with coverage will be assessed using mixed effects logistic regression.

Outcome 4: Community-level confirmed *P. falciparum* malaria parasite incidence

Data pertaining to this outcome will be analyzed on an intention-to-treat basis. Monthly counts of confirmed malaria cases from the health facility registers will be linked to villages and analyzed in a time series Poisson or negative binomial model with random intercepts at the health center catchment and village levels. The models will include a fixed effect for each study arm, an interaction term for the combination of MTAT and FTAT, and a fixed effect for time period (pre- and post-intervention). The interaction between these two terms will be the primary effect measure (also known as the difference-in-differences estimator). Pre/post-intervention will be determined as all time periods before the start date of the intervention in the areas considered as being pre-intervention and all time periods after (and including the start date of the intervention in the area considered as being post-treatment). In addition to a post-intervention analysis of confirmed malaria case incidence from routine health information data, the health facility data will be reviewed monthly to allow near real-time analysis of confirmed and total case outpatient incidence as the three rounds of the MTAT interventions are rolled out across the study areas.

Secondary outcomes

Serology

Seropositivity to a panel of standard malaria parasite antigens for *P. falciparum* and *P. vivax* (including species-specific MSP, AMA, CSP antigens) will be used to examine both recent and medium-term exposures. These data will also be mapped using captured GPS coordinates to examine serological hotspots. Finally, novel antigens will also be used to further explore any differences in parasite exposure between study arms. Vector exposure may also be explored using antigens to anopheline salivary proteins.

Risk factors for malaria parasite infection in HRPs

Survey data, RDT results, and PCR-results will be used to examine economic, demographic, occupational and spatial risk factors for malaria parasite positivity, and identification of specific high-risk sub-populations for interventions. The primary outcomes will be all-species parasite prevalence via RDTs, HS-RDTs (all field testing) plus PCR (cross-sectional surveys, one MTAT round only, and FTAT), combined with surveyed risk factors. Logistic regression will be used to determine risk factors for 1) RDT-based test positivity 2) HS-RDT-based test positivity and 3) PCR-based positivity (cross-sectional surveys, one MTAT round only, and FTAT). Analyses will also be performed for *P. falciparum* and *P. vivax* separately, with appropriate corrections for multiple testing. Models for panel data will be used for cross-sectional surveys (if resample from the same household lists), and for all households with repeat visits during MTAT events. Models will also incorporate robust errors and survey correction factors to address non-independence of observations.

Cost and cost-effectiveness

For the costing portion of the study, the cost per case investigation conducted, cost per additional positive case identified using MTAT in village-based populations and FTAT in forest-based HRP, and the cost per case actively detected per person tested during test and treat. The cost-effectiveness of test and treat will also be compared between village-based activities and peer-navigator led testing. Costing and analysis methods will follow principles of costing for health care programs [27] and analysis and presentation of results will follow the CHEERS guidelines [39]. Costing will be conducted from the health-sector (provider) perspective and will utilize key informant interview and focus group data collection methods.

Sensitivity and specificity of HS-RDTs

The *P. falciparum* prevalence from all individuals tested with HS-RDTs will be compared with parallel results from standard RDTs (all field testing activities) and to subsequent PCR-based testing (cross-sectional surveys only). In the latter case, the PCR-based result will serve as the gold standard for all comparisons. Sensitivity and specificity and ROC analyses will be conducted to assess the performance of the HS-RDTs in field situations, and in the presence of mixed-species infections.

3.3.3 Activity 2 - Assessment of health-sector and community feasibility and acceptability of the test and treat interventions

3.3.3.1 Implementation procedures

Several methods for assessing the feasibility and acceptability of MTAT and FTAT interventions as perceived by communities, HRP, health sector staff, peer navigators, and study staff will be implemented over the course of the study. Peer navigators, while widely employed in other public health domains (e.g., HIV/AIDS, substance abuse, cancer), have yet to be deployed on any scale in the malaria sector. In these settings, peer navigators have successfully engaged hard-to-reach populations, improved health outcomes, and offset cumulative healthcare costs. Given the novel use of peer navigators in a malaria setting, a more in-depth and focused evaluation will be conducted to assess the capacity, feasibility, and acceptability of peer navigators to implement the FTAT intervention. Activity 2 methods are summarized in Table 11 below, and include FGDs, KIIs, PN observations and interviews, and HRP interviews.

Table 11. Methods to assess the feasibility and acceptability of MTAT and FTAT

S/N	Method	Sample Size	Tools/Sources	Sample Data	Timeframe
1	FGDs and KIIs	- 2 FGDs with PNs - 2 FGDs with MTAT team members (8 participants/FGD) - 2 KIIs per district (8 total KIIs)	FGD and KII interview guides (Annex Q, R, S)	- Perceived MTAT and FTAT feasibility and acceptability - MTAT/FTAT barriers and benefits - Study experience (staff and PNs) - Perceived challenges to malaria elimination in Lao PDR	Study end-line
2	Cross-sectional household surveys	- Baseline: 1,120 head of households - End-line: 1,680 head of households	Form 2 (Annex D)	Likert scales (quantitative): - Willingness to participate in MTAT - Benefit of MTAT for household - Benefit of MTAT for community	- Baseline survey - End-line survey
3	Observation of PNs by supervisors	Random subset of 12 PNs	PN competency checklist based on FTAT SOP (Annex U)	FTAT competencies, including: - Adherence to RDT, HS-RDT, and DBS procedures - Adherence to diagnosis, treatment, and referral guidelines	2 observations/PN: 1. First month FTAT 2. Final month FTAT
4	PN interviews	6 PNs per round (random subset)	PN interview guide (Annex V)	PN perceptions over time: - FTAT challenges and barriers - FTAT benefits and facilitators - Performance and self-efficacy - Motivations	3 rounds of interviews: 1. FTAT launch (entrance interview) 2. Midpoint FTAT

				<ul style="list-style-type: none"> - FTAT enabling environment (support and supervision) 	3. Final month FTAT (exit interview)
5	HRP interviews by PN supervisors	Subset of 6-8 HRPs per round	HRP interview guide (Annex W)	<ul style="list-style-type: none"> - Acceptability of PNs and FTAT - Confidence and trust in PNs - Perceived benefits of FTAT - Perceived burden/risk of malaria 	1. First month FTAT 2. Final month FTAT (HRP interviews timed with PN observations)
6	FTAT HRP surveys	2,000 HRP target	Form 9 FTAT Survey (Annex K)	Likert scales (quantitative): <ul style="list-style-type: none"> - Willingness to participate in FTAT - Benefit of FTAT participation 	Continuous during FTAT implementation
7	Analysis of process and output indicators	N/A	Study data and records: <ul style="list-style-type: none"> - FTAT/MTAT survey data - Inventory records - PN call logs/diaries - Lab reports 	<ul style="list-style-type: none"> - # of HRPs surveyed, RDTs, DBS - % HRPs properly treated and referred (compared to MTAT) - PN data completeness and quality (compared MTAT teams) - Supply waste rate (inventory) - # of peer contacts made by PNs - # HRP follow-up calls received - Proportion of collected DBS that can be analyzed by laboratory 	Continuous during study implementation
8	Review of PN meeting agendas and minutes	N/A	PN meeting agendas and minutes	<ul style="list-style-type: none"> - FTAT progress - Challenges and solutions - Refresher training topics - Complaints - PN meeting attendance - Support and supervision 	Bimonthly meetings continuous during FTAT implementation

Notes: PN = peer navigator; HRP = high-risk population; FGD = focus group discussion; KII = key informant interview

3.3.3.2 Primary outcomes

Proportion of village-based survey respondents who strongly disagree, disagree, are ambivalent, agree and strongly agree on the importance and acceptability of community-based MTAT; proportion of surveyed HRPs who strongly disagree, disagree, are ambivalent, agree and strongly agree on the importance and acceptability of FTAT; proportion of peer navigators who rate conducting the FTAT intervention as very easy, somewhat easy, somewhat difficult, and very difficult; proportion of peer navigators receiving a 'pass' score on FTAT competency checklist completed by supervisor during FTAT observation; proportion of peer navigators rating their confidence in conducting the FTAT intervention as very low, low, moderate (confident), and high.

3.3.3.3 Sample size

Focus group discussion (FGD) and key informant interview (KII) activities will aim to reach saturation and be comprehensive. There will be a total of 4 FGDs (2 for PNs, and 2 for MTAT team members), and 2 KIIs per district with district or study staff (8 KIIs total). PN supervisors will observe and complete a FTAT competency checklist (Annex U) on a random subset of 12 PNs at two different time points: within the first month of FTAT launch, and within the final month of FTAT (allowing for pre/post comparison of PN capacity to conduct FTAT and related study interventions). During these two rounds of PN observations, PN supervisors will also interview a subset of 6-8 HRPs (12-16 HRPs total) on the acceptability of PN-FTAT, including confidence and trust in peer navigators, and perceived benefits of FTAT. A random subset of 6 PNs will be interviewed during each round of entrance, mid-FTAT, and exit interviews (18 interviews total) to understand perceived FTAT challenges, facilitators, performance, and self-efficacy. Sample sizes for quantitative (Likert scale) MTAT/FTAT acceptability data derived from cross-sectional and HRP surveys will be based on activity sample sizes: 1,120 households interviewed during baseline, 1,680 households interviewed during end-line, and 2,000 HRPs targeted throughout FTAT implementation.

3.3.3.4 Data collection

Depending on the method, data collection may occur continuously throughout the study, at specific time points, or at end-line only. Standardized tools including interview/discussion guides and a competency checklist will be developed using best practices in qualitative research [40], and translated and back-translated prior to implementation. Study staff and an independent FGD/KII moderator will be trained on the appropriate tools, and all interviews will be conducted in the local language. The end-line FGDs and KIIs will explore constraints and any issues related to all the topics identified from the quantitative survey, field experience implementing the FTAT and MTAT interventions, and barriers to implementation of malaria control efforts. Informed consent will be obtained from all FGD, KII, and HRP participants in the local language prior to any data collection. FGDs and KIIs will be audio-recorded, transcribed, and the transcripts translated into English.

3.3.3.5 Analytic plan

Quantitative analysis will consist of comparison of the proportions of different study respondents having a favorable view of MTAT and FTAT interventions, and identification of any socio-demographic or occupational groups that have significantly different distributions of responses from the overall study population.

Grounded theory will be used to inform data coding [40], which will be performed by a study analyst(s) using dedicated qualitative analysis software (NVivo, ATLAS.ti, or TAMS Analyzer). The grounded theory method relies on a constant comparative approach to generate theories about human behavior. Interview transcripts or detailed notes are coded into concepts reflecting the objectives of the assessment, and identified concepts are grouped into categories, which are then organized into broader themes. Relationships among analytic categories or themes are used to interpret the data and generate explanations about human actions and behaviors. Analysis of the study data will focus on identification of any practical or intervenable barriers to FTAT or MTAT implementation and uptake to inform future practice and policy.

3.3.4 Activity 3 - Estimating population size of forest-based HRPs

3.3.4.1 Implementation procedures

During all PN-led FTAT activities, all persons who provide informed consent will be asked several survey questions to help estimate the population size of HRPs in the HCCA area.

1. After identification of possible HRPs at any non-village sites, PNs will assess their eligibility, and proceed through the informed consent process.
2. Several questions will ask the respondents to estimate the total number of persons like themselves (using sex, plus linguistic, social, and occupation-based criteria) within the immediate area (5-10 km radius).

For the second type of population estimation, data collected at each HRP contact event (mobile phone numbers, age or date of birth, home village, and initials) will allow for generation of a unique and reproducible personal identifier. At the end of the study period, the unique personal identifier codes will be used to estimate the HRP population size using single-source capture-recapture methods [41], complemented with double-list capture recapture methods.

3.3.4.2 Primary outcomes

The primary outcome will be three separate population size estimates with appropriate confidence intervals.

3.3.4.3 Sample size

There are no formal sample size calculations for “wisdom of the crowds” methods [42] as this method is based on the assumption that taken in aggregate, members of a target population can plausibly estimate the total number of persons like themselves.

The sample size for the single source capture-recapture uses simulations from a Poisson distribution to ensure useful limits for the confidence intervals in the population size estimates. With an assumed total HRP population of 5,000 persons, if 750 are enrolled in the study, 95% confidence interval for the population size estimate will be 2,300 to 7,700; larger sample sizes will have correspondingly narrower CIs.

For the double-source capture recapture using lists generated by the PN teams working in the same HCCA, with an assumption of 2,000 persons surveyed and with a 4% recapture rate (and therefore approximately 145 per HCCA), the confidence interval for the population size per HCCA will be sufficiently narrow at 213 (95% CI: 198 to 231), or 2,982 (95% CI: 2,772 - to 2,982) for the pooled HCCAs.

3.3.4.4 Data collection

Data collection for sample size estimates will occur during all peer-navigator led FTAT activities. Essential demographics will be collected on all persons identified as forest-goers by PNs during their routine activities, allowing for generation of a unique and reproducible personal identifier for all HRPs.

3.3.4.5 Analytic plan

Three separate estimates for the total HRP population in the target areas will be produced and compared. Standard methods for “wisdom of the crowds” [42] and two-source capture recapture [43] will be utilized. The methods estimating population size from a single-source capture recapture list will use truncated Poisson and related models with inclusion of relevant covariates [42,43] where maximum likelihood fit distribution are used to infer the hidden ‘zero’ capture population size.

The second capture-recapture analysis will be performed by comparing lists between the two different PN teams that have been operating in the same HCCA using standard multiple-list methods for multiple-observer surveys [43,46]. As the transient nature of many HRPs may violate a key assumption of capture-recapture studies (no in or out migration of the study population) data will be analyzed by individual study month to minimize this bias. As a sensitivity analysis, the full annual data will also be analyzed using alternative models which allow for moderate migrations to be assessed [47,48].

A third capture-recapture analysis will use the unique identifiers captured during PN activities and numbers of those individuals reporting living in a nearby village, compared with proportions of all household members reporting forest-going or forest-fringe farming activities during baseline and MTAT surveys, to estimate the total size of the HRP population (and sub-populations wherever feasible).

3.3.5 Activity 4 - Assess demographic and geographic predictors for G6PDd testing adherence with a positive *P. vivax* RDT

3.3.5.1 Implementation procedures

During all MTAT and FTAT RDT testing activities, all patients with a *P. vivax* positive RDT (both febrile and asymptomatic) will be given a different coded and signed informational letter directing them to the nearest district hospital for G6PDd testing and possible radical cure depending on results. Study contact information will also be included if the participants present at other health facilities.

3.3.5.2 Primary outcomes

The primary outcome will be the proportion of referred patients who present to district hospitals for G6PDd testing.

3.3.5.3 Sample size

In 2016, there were about 100 confirmed *P. vivax* cases per district per month in Champasak. If 30% of referred patients present for testing, then the study will be adequately powered (80%; at 5% significance level) to examine 10 risk factors if at least 500 persons are referred [49].

3.3.5.4 Data collection

As part of the normal data collection activities for MTAT and FTAT, all patients with an RDT positive for *P. vivax* mono- or mixed infection will receive an individually coded letter directing them to the nearest district hospital for G6PD testing.

The study staff will meet with district hospital staff (monthly) from all study area district hospitals and all nearby district hospitals to collect these forms, with small incentives for health staff to report receiving a referral letter (phone top-up card, etc.) to maximize data completeness.

3.3.5.5 Analytic plan

At the conclusion of the study, data captured from the total distributed and returned G6PD testing referral letters will be linked to individual records from household or forest-based surveys.

Logistic regression will be used to examine risk factors for non-adherence to testing with 'presented for G6PDd testing' as the outcome. Covariates to be included include distance from the village to district hospital, fever status, occupational factors, and demographic characteristics. These models will be used to explore the factors associated with adherence for G6PD testing referral among both asymptomatic and febrile patients.

4 Ethical issues related to human subject research

4.1 Adequacy of protection against risks

We will administer an informed consent form both verbally and in writing to all participants in the local language for participation in the cross-sectional surveys, MTAT interventions, and FTAT activities. These forms will be read or will be given to participants to read themselves and will include a full description of voluntary participation, the right to withdraw from the study at any time, and the right to not answer any question or participate in any component of the research.

These forms will also address the risks, benefits, and purpose of the study and what we hope to learn, with a specific focus on the potential risks associated with the administration of SLD-PQ. We will train

all interviewers extensively on the consent procedure, and each form will be co-signed by a team member to ensure all participants have consented. Checks in the field by the PI and project leaders will further ensure the consent process is followed in all cases. Data collection team members will provide the contact information for study coordinators who can be contacted for any further information on the topics brought up in the interview, or for additional treatment if necessary. The confidentiality procedures are designed to meet all contingencies to ensure the confidentiality of participant data and the privacy of the participants is preserved.

Our proposed strategies to reduce risks to privacy or of disclosure of confidential information include:

1. Identifying information will be recorded only in secure database software on password protected computers, and data collectors will only have access to the data that they themselves directly collect which will be cleared from their devices after all follow-up visits are completed. All data will be stored only in password-protected files on password-protected computers in locked offices.
2. Prior to analysis, data will be de-identified with the exception of geo-location codes, which are necessary for specific per-protocol analyses. The absence of individual identifying information will protect subject confidentiality.
3. All paper records will be stored in a locked location.

4.2 Protection against risks associated with the administration of AL / SLD-PQ

Artemether lumefantrine (AL) is standard of care for uncomplicated malaria in Lao PDR. The use of SLD-PQ for *P. falciparum* [16] has been shown to have no clinically significant impacts on hematological indexes in populations with severe G6PD deficiencies in the GMS [22], and no SAEs are expected from SLD-PQ.

The key to managing side effects and any potential adverse events (AEs) or serious AEs (SAEs) is thorough training of VMWs/VHVs, health facility staff and supervisors, and intensive sensitization of the community. HC workers and VMWs/VHVs will passively monitor their respective communities for AEs and refer any potential SAEs to designated health facilities. Health workers at these facilities will be informed of the project and potential side effects, and undergo training on completion of AE reporting forms (Appendix N). Cases that cannot be treated at health centers will be referred to district or provincial hospitals.

All participants receiving study drug will be provided with an informational sheet listing potential side effects and instruction to seek care at the nearest health center should they experience any of the defined symptoms or other adverse events. Community sensitization events and targeted IEC/BCC materials will also help increase community awareness of potential adverse events and encourage early care-seeking if events arise.

A passive case detection system will be employed in both intervention and control villages to help detect and refer any potential SAEs; VMWs/VHVs will be oriented on potential drug side effects and asked to passively monitor their communities and refer any potentially serious or unexpected AEs. All public health facilities in the four target districts will be trained on AE reporting procedures prior to implementation of any study activities. A data safety monitoring board (DSMB) will be established to oversee and report on any SAEs that are potentially linked to the administration of AL or SLD-PQ, as outlined in further detail in Section 4.5. The following steps will be taken for all AEs:

The health facility will complete an AE reporting form (Appendix N). The completed form will then be submitted to the field manager (a medical doctor), who with the support of the UCSF project manager will assess if the event is an SAE or unexpected AE based upon specific criteria determined by the DSMB. If determined to be an SAE or unexpected AE, the UCSF project manager will submit the AE reporting form and any accompanying documentation (clinical records, laboratory reports) to the DSMB and the UCSF PI within 48 hours from the identification of the potential SAE.

Upon receipt of the AE reporting form, the DSMB will complete an AE investigation form (Appendix O) within 24 hours to a) confirm if the event is an SAE, and 2) to determine if the SAE was caused by the administration of AL with SLD-PQ as part of this study. If the event is confirmed to meet the criteria of an SAE, the DSMB will submit the completed AE investigation form to the PI, who will submit to the research ethics committees (RECs) at UCSF and NIOPH within 24 hours of receipt from the DSMB.

4.3 Potential benefits of the proposed research to the participants and others

The proposed research may benefit patients in direct and indirect ways. Participants will directly benefit from detection of low-level parasitemia, and the curative effects of AL administration on existing *P. falciparum* infections in both the cross-sectional, MTAT, and FTAT interventions. Furthermore, patients may directly benefit due to community-wide reductions in malaria transmission that are expected to occur after the application of the interventions. Finally, entire target village populations will benefit from SLD-PQ, which targets the parasite sexual stages thereby decreasing overall transmission.

Participants may also indirectly benefit, as the information gained from this research will be used to help establish the safety and efficacy of a new malaria control intervention in Lao PDR. The research will benefit the scientific and malaria control communities more generally by expanding the evidence base on MTAT, FTAT, and addition of gametocytocidal drugs to treatment regimens in the GMS.

4.4 Alternatives to participation

Participation in the research study is voluntary. Individuals electing not to participate in the research study may still receive testing and treatment as part of the interventions. Individuals who do not wish to receive testing and treatment during the intervention campaigns may visit local health facilities for malaria testing and treatment.

4.5 Data and safety monitoring plan

The project will follow US National Institutes of Health (NIH) guidelines for establishing a data safety monitoring board (DSMB). The DSMB will be established prior to any data collection as part of this study. The members of the DSMB will serve in an individual capacity and provide their expertise and recommendations. The primary responsibilities of the DSMB will be to periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy, and 2) make recommendations to investigators concerning the continuation, modification, or termination of the trial. DSMB will consider study-specific data as well as relevant background knowledge about malaria, adverse events from AL and SLD-PQ, drug resistance, and the participant population under study.

Membership of the DSMB will consist of five independent experts in malaria control, diagnosis, case management and epidemiology, and one member of the research team to advise and clarify study activities for the independent experts. No independent member of the DSMB shall have any conflict of interest with the study team, the organizations funding or conducting the research, or the results of the

study. The DSMB will be comprised of experts in the following areas, with an emphasis on local Lao expert participation:

- The study population in Southern Lao PDR (Lao representative)
- Malaria diagnosis and case management
- Malaria epidemiology
- Biostatistics
- Conduct of clinical trials
- Malaria drug resistance

No data on futility or benefit of the intervention will be estimable during the course of the trial as the timing of outcome data collection precludes developing stopping rules based on outcome data collected during implementation. Safety concerns associated with the wide-scale use of AL + SLD-PQ, although unexpected, will form the basis of development of a stopping rule. The stopping rules for this trial will be based on detection of a significantly higher rate of mortality, hospitalization for possible drug-related events, or any other SAE in the MTAT villages versus rates recorded at health facilities for the control (non-MTAT) villages. Further, any investigated SAE that results in death that is found to be due to the administration of AL + SLD-PQ will be grounds for stoppage.

4.6 Collection of specimens

RDT kits for malaria using finger prick blood samples will be collected during the research and intervention. These samples and their byproducts will be collected for disposal according to local disposal standards for biohazard and sharps waste at the nearest health facility (generally incineration). DBS will also be collected for PCR analysis of malaria parasite infection. These blood spots will be disposed of after the research and not stored for future use. The research period will be defined by the approval dates for the local research ethics committee, the UCSF, and CMPE, and blood samples will not be stored for any longer than three years past the date the research study end. The informed consent documents will specify the uses and storage plans for dried blood specimens. Patient identifying information will be replaced with an unrelated unique identifier on each filter paper. All appropriate universal and site-specific safety precautions will be used in handling sharps, RDTs, microscopy slides, and filter paper blood spots. All survey workers will be trained in the proper storage and handling of blood samples prior to fieldwork.

4.6.1 Racial and ethnic origin

A diverse range of ethnic groups will be included in the study, including several marginalized ethnic minorities. Our study will be conducted outside of the U.S., and no racial/ethnic group will be excluded. We do not expect to find race/ethnicity differences in the intervention effect, but refusal rates may differ.

4.6.2 Inclusion of vulnerable subjects – children and pregnant women

All age groups will be included in this study, except for those < 18 months. All children that participate must have the consent of the parent or guardian. Children older than 6 years and less than 18 must also provide oral assent before participation. All women who are pregnant or believe they may be pregnant will be assessed as in section 3.2.1.

4.7 Subject consent/assent

4.7.1 Process of consent

Informed, written consent will be sought from all participants in the study. Individuals 18 years of age and older will provide individual consent. For individuals >18 months and less than 18 years of age, consent will be sought from the head of household or parent/household guardian at least 18 years of age. For children greater than 6 years and less than 18, oral assent will be sought from the child.

4.7.2 Subject capacity

For individuals who do not have the capacity to provide consent for testing and treatment, consent will be sought from the head of household or parent/guardian. If either is absent during the household visit, the subject without capacity to provide individual consent will be excluded. If individuals are unable to sign consent forms, they may make a thumbprint mark to indicate consent.

4.7.3 Subject/representative comprehension

All consent forms will be read in local languages to individuals in the study areas, and provided in Lao in written form.

4.7.4 Documentation of consent

Written consent will be obtained from all participants as described above in 4.1 and maintained in Vientiane.

4.7.5 Costs to the subject

There are no costs to the subject for participation in any project activities.

4.7.6 Payment for participation

No payment for participation will be provided to participants. Households for the cross-sectional surveys will receive a small gift for participation (e.g., soap bars), and forest-based HRPs will receive a torch or other small token of appreciation.

4.7.7 Field data collector training

All VMWs/VHVs and health professionals recruited from CMPE and NIOPH to implement the study interventions will receive standardized training on the process of informed consent, the protection of the privacy of study subjects, the protection of the confidentiality of data pertaining to study subjects, and in safe and proper methods for conduct of finger sticks for malaria parasite testing. Every effort will be made to prevent secondary infection from the finger stick by using new, sterile lancets for each individual tested and by cleaning the finger with an alcohol swab prior to conducting the finger stick. HC workers and VMWs/VHVs will be provided with sufficient supplies for this throughout the fieldwork. In addition, the field staff will be provided with and will wear a fresh pair of latex gloves for each individual receiving a finger stick. The first drop of blood will be wiped from the finger; the second-third drops will be used for RDT-based tests, and where applicable, the four-seventh collected on filter paper for later analysis.

Health staff throughout the project area will also receive dedicated training on best practices in facility-level data collection, and regular supportive supervision to ensure data quality and completeness throughout the study period by district project coordinators. These trainings will also include use of SOPs for the reporting of any AEs or SAEs from within the catchment areas.

4.7.8 Community sensitization and communication

To prepare communities for intervention roll-out and survey data collection, community sensitization and engagement activities will be implemented. Prior work in the GMS has highlighted the critical role of these activities in successful implementation of drug-based approaches at the community level [50]. Specifically, the need for local health staff and community leaders to be the primary conveyors of study information (as opposed to study staff) is a critical component of high levels of community participation.

Included in this package will be a general informational letter and accompanying flyer for districts and local communities. These documents will include information about the purpose, procedures, and the importance of household participation. Meetings will be coordinated with the local DAMNs and among local community leaders in advance of the training and campaign activities, and in addition to the meetings with health facility and community health workers.

4.7.9 Study organization and management

The study will be under the overall management of CMPE with oversight being provided by the MOH. Implementation and general management of the various activities will be done by CMPE and NIOPH, with UCSF providing programmatic and technical support. Administratively, health services are decentralized in Lao PDR and therefore the DAMNs in Champasak Province will provide supervisory oversight at district level.

5 Potential risks, limitations, data quality assurance, and dissemination plan

5.1 Potential risks from participation

Detailed discussion of potential AEs related to drug regimens is discussed above in section 4.2. Finger pricks for RDTs and DBS are associated with small risks of bleeding, hematoma, and infection. To minimize these risks, the skin will be cleaned with alcohol prior to puncture, and sterile unused lancets will always be used, and pressure will be placed on the puncture site after removal of the lancet using sterile gauze. Although the quantity of blood drawn would not lead to any ill effects on the participants' health, some adults and rarely children feel faint from the blood during the finger prick. The risks will be minimized by having trained health staff perform all procedures, and all untoward effects will be evaluated by health center staff.

5.2 Limitations

Several limitations have the potential to compromise study outcomes.

Malaria declines

There may be large-scale changes in malaria incidence and prevalence throughout the study area over the course of the 12-month implementation period, which could compromise study power.

Malaria increases

Any large increases in malaria burden or other diseases (e.g., dengue) in target districts could increase overall caseloads at health centers, potentially impacting availability of VMWs/VHVs or other staff to support test and treat campaigns, especially in areas with ethnic minorities where their expertise is crucial.

Widespread HRP2/3 deletions

There have been no surveys for *P. falciparum* HRP2/3 deletions in Lao PDR, and only limited data from other settings in the GMS: in China and Myanmar deletions were detected in 4/87 samples (unpublished

data WHO MPAC, 2017). If these deletions are common in the study sites [51], the impact of HRP2-based test and treat could be severely compromised. *P. falciparum* positive DBS from the baseline cross-sectional survey will be rapidly analyzed to assess the level of HRP2/3 deletions in Southern Lao PDR.

Changes in forest-going activity patterns

The Lao government decree (May 2016) is believed to have had a major impact on the total number of illegal and semi-legal forest-goers. However, the future status is unknown; if there are changes to the decree itself or to its enforcement, the PNs could potentially be overwhelmed with interviews or sampling sites to target.

Mobility of target populations

The fluxional nature of HRPs targeted in this intervention and the mobility inherent in forest-based economic activities has the potential to contaminate the HCCA-level randomization. While HRPs may freely transit between intervention and non-intervention arms, and thereby ‘carry-over’ PN-led interventions, the use of unique IDs will allow the impact of these movements to be assessed and adjusted for in exploratory analyses.

Other partners’ interventions impact study outcomes

The implementation of diverse programs by other partners across the study area has the potential to impact study outcomes if implementation is differential across the study arms. The nature of the randomization should minimize biases from partner activities, and a detailed matrix of other project activities will be created, and used to assess and adjust outcomes for exploratory analyses.

5.3 Data quality assurance plan

Data quality and management

Data collection will occur in multiple locations, and differences in data collection systems may exist at different locations, which could potentially bias results. However, study teams and regular health staff will receive comprehensive training, as well as ongoing evaluation, supervision, and supplementary capacity building as necessary to ensure data quality and completeness.

Procedures to minimize biases

A survey instrument based on the formative work (carried out in December 2016) will be developed in English with input from collaborators at CMPE. This will then be translated to Lao and back-translated by a fluent bilingual health expert before field testing. Any ethnic minority language interviews will be conducted in the appropriate language, and translated into Lao. Formative work will assess the feasibility of using tablets for data entry versus paper questionnaires. A pilot study will test the utility of the survey instruments; these data will then be discarded if significant changes are made to the survey instrument. Study coordinators will be responsible for monitoring data quality to ensure that questionnaires are completed and entered correctly.

Potential changes to this protocol based on the piloting of tools and methods

The organization and supervision of the peer navigators may be changed based on initial feasibility studies during field-testing of survey instruments.

5.4 Dissemination Plan

The finalized protocol will be filed at <http://www.clinicaltrials.gov/> prior to implementation, and will conform to CONSORT recommendations for cluster randomized trials [52].

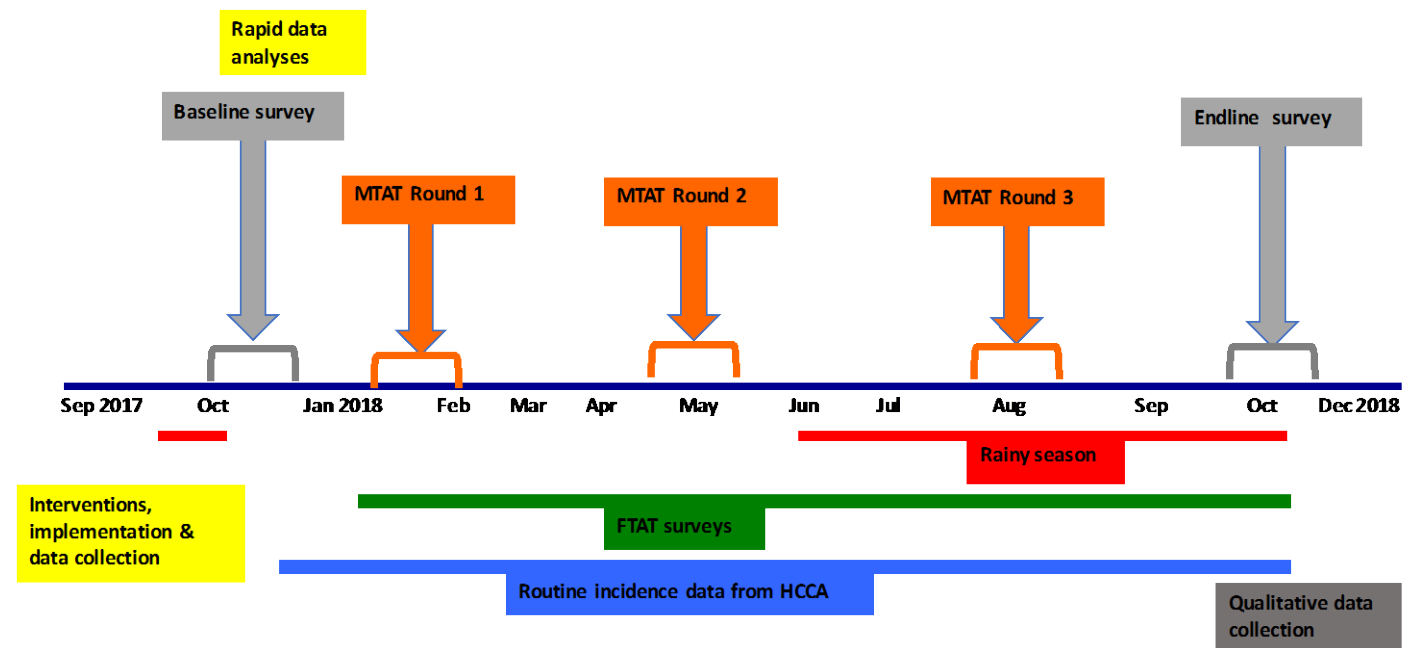
The results of the baseline survey, as well ‘hotspots’ identified during village-level MTAT and peer-navigator led FTAT activities will be shared on a real-time basis with national, provincial, district and village-level partners as well as other key stakeholders (i.e., WHO, CHAI, PSI) throughout implementation to ensure that the most up-to-date information about malaria is available in the target districts.

6 Budget

No.	Description	Total
A	START-UP OPERATIONS	
1	IRB Application	2,000,000
2	Pilot testing (early August 2017)	18,571,429
SUBTOTAL START-UP OPERATIONS		20,571,429
B	TRAININGS AND PROJECT ORIENTATION	
1	Core study team training (3 days in Vientiane)	11,350,000
2	Project orientation (1 day in Pakse)	41,548,571
3	Cross-sectional survey training - (3 days in Pakse following orientation - November 2017)	28,364,000
4	Health facility staff training on A3 and AE reporting, G6PD (2 days in Pakse following cross-sectional training)	55,660,000
5	PN training (4 days in Pakse - December 2017)	71,194,286
6	MTAT training (4 days in Pakse - January 2017)	67,610,286
SUBTOTAL TRAININGS AND PROJECT ORIENTATION		275,727,143
C	CROSS-SECTIONAL SURVEYS AND INTERVENTIONS	
1	Baseline survey (HH survey, RDT, HS-RDT, and DBS/PCR)	105,900,000
2	Endline survey (HH survey, RDT, HS-RDT, DBS/PCR, and qualitative FGDs/KIIs)	105,900,000
3	MTAT staff (3 rounds x 30 days/round = 90 days)	379,400,000
SUBTOTAL CROSS-SECTIONAL SURVEY AND INTERVENTIONS		591,200,000
D	EQUIPMENT AND SUPPLIES	
1	Vehicle, equipment, and supplies	28,000,000
2	Communications, community sensitization and IEC/BCC materials	42,720,000
SUBTOTAL EQUIPMENT AND SUPPLIES		70,720,000
E	SUPERVISION AND MONITORING	
1	Supervision and monitoring visit by MoH (DCDC, CMPE and NIOPH)	67,771,429
SUBTOTAL SUPERVISION AND MONITORING		67,771,429
Grand total		1,025,990,000

7 Timeline

Table 9. Preliminary timeline for project activities



8 References

1. Tiono AB, Ouédraogo A, Ogutu B, Diarra A, Coulibaly S, Gansané A, et al. A controlled, parallel, cluster-randomized trial of community-wide screening and treatment of asymptomatic carriers of *Plasmodium falciparum* in Burkina Faso. *Malar J.* 2013;12: 79.
2. Mosha JF, Sturrock HJ, Greenhouse B, Greenwood B, Sutherland CJ, Gadalla N, et al. Epidemiology of subpatent *Plasmodium falciparum* infection: implications for detection of hotspots with imperfect diagnostics. *Malar J.* 2013;12: 221. doi:10.1186/1475-2875-12-221
3. Cook J, Xu W, Msellem M, Vonk M, Bergstrom B, Gosling R, et al. Mass screening and treatment on the basis of results of a *Plasmodium falciparum*-specific rapid diagnostic test did not reduce malaria incidence in Zanzibar. *J Infect Dis.* 2015;211: 1476–1483. doi:10.1093/infdis/jiu655
4. Hustedt J, Canavati SE, Rang C, Ashton RA, Khim N, Berne L, et al. Reactive case-detection of malaria in Pailin Province, Western Cambodia: lessons from a year-long evaluation in a pre-elimination setting. *Malar J.* 2016;15: 132. doi:10.1186/s12936-016-1191-z
5. Parker DM, Landier J, von Seidlein L, Dondorp A, White L, Hanboonkunupakarn B, et al. Limitations of malaria reactive case detection in an area of low and unstable transmission on the Myanmar–Thailand border. *Malar J.* 2016;15: 571. doi:10.1186/s12936-016-1631-9
6. Hoyer S, Nguon S, Kim S, Habib N, Khim N, Sum S, et al. Focused screening and treatment (FSAT): a PCR-based strategy to detect malaria parasite carriers and contain drug resistant *P. falciparum*, Pailin, Cambodia. *PLoS ONE.* 2012;7: e45797. doi:10.1371/journal.pone.0045797
7. Cotter C, Sturrock HJ, Hsiang MS, Liu J, Phillips AA, Hwang J, et al. The changing epidemiology of malaria elimination: new strategies for new challenges. *The Lancet.* 2013;382: 900–911. doi:10.1016/S0140-6736(13)60310-4
8. World Health Organization. Strategy for malaria elimination in the Greater Mekong Subregion: 2015-2030 [Internet]. Geneva: Global Malaria Program, WHO; 2015. Available: http://iris.wpro.who.int/bitstream/handle/10665.1/10945/9789290617181_eng.pdf
9. World Health Organization Regional Office for South-East Asia (SEARO). Approaches for mobile and migrant populations in the context of malaria multi-drug resistance and malaria elimination in the Greater Mekong Subregion [Internet]. Thailand; 2016. Available: http://apps.who.int/iris/bitstream/10665/204351/2/Approaches_%20mobile_migrant_populations.pdf
10. Center for Malariology, Parasitology, and Entomology, Lao PDR. Lao PDR Malaria National Strategic Plan 2016-2020. Vientiane, Lao PDR: Ministry of Health, Lao PDR; 2015.

11. Edwards HM, Canavati SE, Rang C, Ly P, Sovannaroeth S, Canier L, et al. Novel cross-border approaches to optimise identification of asymptomatic and artemisinin-resistant *Plasmodium* infection in mobile populations crossing Cambodian borders. PLOS ONE. 2015;10: e0124300. doi:10.1371/journal.pone.0124300
12. Khamsiriwatchara A, Wangroongsarb P, Thwing J, Eliades J, Satimai W, Delacollette C, et al. Respondent-driven sampling on the Thailand-Cambodia border. I. Can malaria cases be contained in mobile migrant workers? Malar J. 2011;10: 120. doi:10.1186/1475-2875-10-120
13. Wangroongsarb P, Hwang J, Thwing J, Karuchit S, Kumpetch S, Rand A, et al. Using respondent driven sampling to identify malaria risks and occupational networks among migrant workers in Ranong, Thailand. PLOS ONE. 2016;11: e0168371. doi:10.1371/journal.pone.0168371
14. Briand V, Le Hesran J-Y, Mayxay M, Newton PN, Bertin G, Houzé S, et al. Prevalence of malaria in pregnancy in southern Laos: a cross-sectional survey. Malar J. 2016;15: 436. doi:10.1186/s12936-016-1492-2
15. Makanga M. A review of the effects of artemether-lumefantrine on gametocyte carriage and disease transmission. Malar J. 2014;13: 291. doi:10.1186/1475-2875-13-291
16. Dicko A, Brown JM, Diawara H, Baber I, Mahamar A, Soumare HM, et al. Primaquine to reduce transmission of *Plasmodium falciparum* malaria in Mali: a single-blind, dose-ranging, adaptive randomised phase 2 trial. Lancet Infect Dis. 2016; doi:10.1016/S1473-3099(15)00479-X
17. Gerardin J, Eckhoff P, Wenger EA. Mass campaigns with antimalarial drugs: a modelling comparison of artemether-lumefantrine and DHA-piperaquine with and without primaquine as tools for malaria control and elimination. BMC Infect Dis. 2015;15: 144. doi:10.1186/s12879-015-0887-y
18. Maude RJ, Socheat D, Nguon C, Saroth P, Dara P, Li G, et al. Optimising strategies for *Plasmodium falciparum* malaria elimination in Cambodia: primaquine, mass drug administration and artemisinin resistance. PLoS ONE. 2012;7: e37166. doi:10.1371/journal.pone.0037166
19. Corrigan PW, Pickett S, Batia K, Michaels PJ. Peer navigators and integrated care to address ethnic health disparities of people with serious mental illness. Soc Work Public Health. 2014;29: 581–593. doi:10.1080/19371918.2014.893854
20. AIDS United. Best practices for integrating peer navigators into HIV models of care [Internet]. Washington, D.C.: AIDS United; 2015 May. Available: https://www.aidsunited.org/data/files/Site_18/PeerNav_v8.pdf

21. Health Poverty Action. Formative assessment to identify mobile and migrant population networks in Southern Lao considered at-risk for malaria infection- Final report. Vientiane, Lao PDR: Health Poverty Action; 2016.
22. Bancone G, Chowwiwat N, Somsakchaicharoen R, Poodpanya L, Moo PK, Gornasawun G, et al. Single low dose primaquine (0.25mg/kg) does not cause clinically significant haemolysis in G6PD deficient subjects. PLOS ONE. 2016;11: e0151898.
doi:10.1371/journal.pone.0151898
23. Center of Malariology, Parasitology, and Entomology, Ministry of Health, Lao PDR. Guidelines for the diagnosis and treatment of uncomplicated malaria for health centers and village health volunteers/workers. Vientiane, Lao PDR; 2013 Jul p. 19.
24. World Health Organization. Guidelines for the treatment of malaria [Internet]. 3rd ed. Geneva: World Health Organization; 2015. Available:
<http://www.ncbi.nlm.nih.gov/books/NBK294440/>
25. Leang R, Khu NH, Mukaka M, Debackere M, Tripura R, Kheang ST, et al. An optimised age-based dosing regimen for single low-dose primaquine for blocking malaria transmission in Cambodia. BMC Med. 2016;14. doi:10.1186/s12916-016-0701-8
26. UCSF, Malaria Center, LSHTM. Single low-dose primaquine to Interrupt *P. falciparum* transmission in Africa: A Roadmap Update: Meeting Summary [Internet]. London; 2014 Mar. Available:
<http://globalhealthsciences.ucsf.edu.s116768.gridserver.com/sites/default/files/content/ghg/mei-london-pq-summary.pdf>
27. Drummond MF, Sculpher MJ, Torrance GW, J. O, Stoddart GL. Methods for the Economic Evaluation of Health Care Programmes [Internet]. Oxford University Press; 2005. Available:
<http://econpapers.repec.org/bookchap/oxpobooks/9780198529453.htm>
28. Torgerson DJ, Torgerson CJ. Cluster Randomised Controlled Trials. Designing Randomised Trials in Health, Education and the Social Sciences. Palgrave Macmillan UK; 2008. pp. 99–107. doi:10.1057/9780230583993_9
29. Mcgee D. Split Plot Designs. In: Armitage P, Colton T, editors. Encyclopedia of Biostatistics. Chichester, UK: John Wiley & Sons, Ltd; 2005. doi:10.1002/0470011815.b2a16073
30. Altman N, Krzywinski M. Points of significance: Split plot design. Nat Methods. 2015;12: 165–166.
31. Gibson DG, Ochieng B, Kagucia EW, Were J, Hayford K, Moulton LH, et al. Mobile phone-delivered reminders and incentives to improve childhood immunisation coverage and timeliness in Kenya (M-SIMU): a cluster randomised controlled trial. Lancet Glob Health. 2017;5: e428–e438.

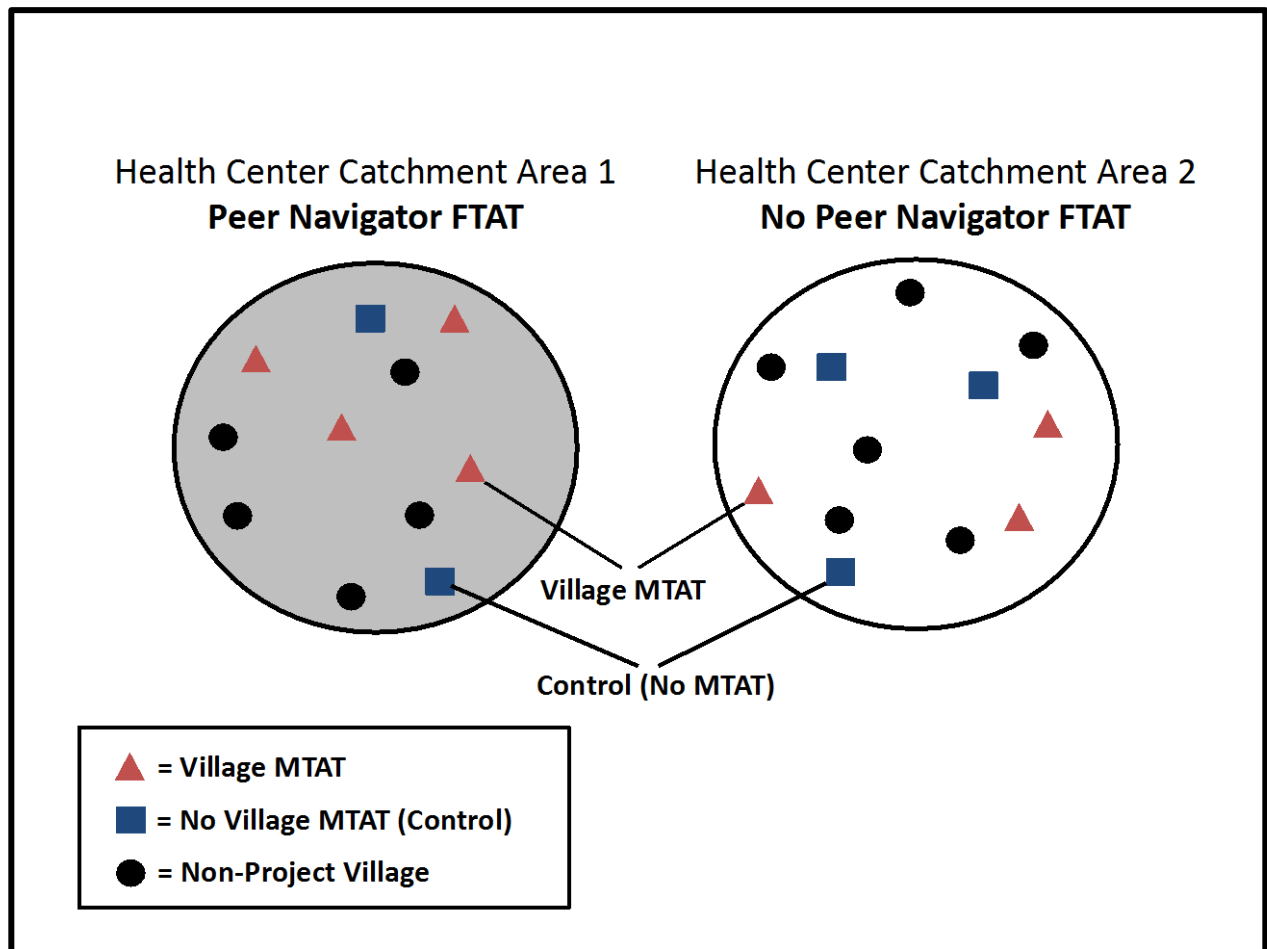
32. Arnold BF, Hogan DR, Colford JM, Hubbard AE. Simulation methods to estimate design power: an overview for applied research. *BMC Med Res Methodol*. 2011;11: 94.
33. R Core Team. R: A language and environment for statistical computing [Internet]. Vienna, Austria: Foundation for Statistical Computing; 2013. Available: <http://www.R-project.org/>
34. Johnson PCD, Barry SJE, Ferguson HM, Müller P. Power analysis for generalized linear mixed models in ecology and evolution. *Methods Ecol Evol*. 2015;6: 133–142. doi:10.1111/2041-210X.12306
35. Canier L, Khim N, Kim S, Sluydts V, Heng S, Dourng D, et al. An innovative tool for moving malaria PCR detection of parasite reservoir into the field. *Malar J*. 2013;12: 405. doi:10.1186/1475-2875-12-405
36. Baum E, Sattabongkot J, Sirichaisinthop J, Kiattibutr K, Davies DH, Jain A, et al. Submicroscopic and asymptomatic *Plasmodium falciparum* and *Plasmodium vivax* infections are common in western Thailand - molecular and serological evidence. *Malar J*. 2015;14: 95. doi:10.1186/s12936-015-0611-9
37. Corran P, Coleman P, Riley E, Drakeley C. Serology: a robust indicator of malaria transmission intensity? *Trends Parasitol*. 2007;23: 575–582.
38. Chan CW, Sakihama N, Tachibana S-I, Idris ZM, Lum JK, Tanabe K, et al. *Plasmodium vivax* and *Plasmodium falciparum* at the crossroads of exchange among islands in Vanuatu: implications for malaria elimination strategies. *PLoS ONE*. 2015;10: e0119475. doi:10.1371/journal.pone.0119475
39. Husereau D, Drummond M, Petrou S, Carswell C, Moher D, Greenberg D, et al. Consolidated health economic evaluation reporting standards (CHEERS) statement. *BMJ*. 2013;346: f1049–f1049. doi:10.1136/bmj.f1049
40. Ulin PR, Robinson ET, Tolley EE. *Qualitative Methods in Public Health: A Field Guide for Applied Research*. 1st ed. Jossey-Bass; 2004.
41. Hay G, Smit F. Estimating the number of drug injectors from needle exchange data. *Addict Res Theory*. 2003;11: 235–243. doi:10.1080/1606635031000135622
42. Okal J, Geibel S, Muraguri N, Musyoki H, Tun W, Broz D, et al. Estimates of the size of key populations at risk for HIV infection: men who have sex with men, female sex workers and injecting drug users in Nairobi, Kenya. *Sex Transm Infect*. 2013;89: 366–371. doi:10.1136/sextrans-2013-051071
43. Hook EB, Regal RR. Capture-recapture methods in epidemiology: methods and limitations. *Epidemiol Rev*. 1995;17: 243–264.

44. Böhning D, Suppawattanabodee B, Kusolvisitkul W, Viwatwongkasem C. Estimating the number of drug users in Bangkok 2001: A capture–recapture approach using repeated entries in one list. *Eur J Epidemiol.* 2004;19: 1075.
45. Böhning D, van der Heijden PGM. A covariate adjustment for zero-truncated approaches to estimating the size of hidden and elusive populations. *Ann Appl Stat.* 2009;3: 595–610. doi:10.1214/08-AOAS214
46. Archaux F. Could we obtain better estimates of plot species richness from multiple-observer plant censuses? *J Veg Sci.* 2009;20: 603–611. doi:10.1111/j.1654-1103.2009.01079.x
47. Hook EB, Regal RR. Accuracy of alternative approaches to capture-recapture estimates of disease frequency: internal validity analysis of data from five sources. *Am J Epidemiol.* 2000;152: 771–779. doi:10.1093/aje/152.8.771
48. Ledberg A, Wennberg P. Estimating the size of hidden populations from register data. *BMC Med Res Methodol.* 2014;14: 58. doi:10.1186/1471-2288-14-58
49. Rothman KJ, Greenland S, Lash TL. *Modern epidemiology.* Third ed. Philadelphia: Lippincott Williams & Wilkins; 2008.
50. Pell C, Tripura R, Nguon C, Cheah P, Davoeung C, Heng C, et al. Mass anti-malarial administration in western Cambodia: a qualitative study of factors affecting coverage. *Malar J.* 2017;16: 206. doi:10.1186/s12936-017-1854-4
51. World Health Organization. False-negative RDT results and implications of new reports of *P. falciparum* histidine-rich protein [Internet]. Geneva: Global Malaria Program, WHO; 2016. Report No.: WHO/HTM/GMP/2016.4. Available: <http://apps.who.int/iris/handle/10665/208819>
52. Campbell MK, Piaggio G, Elbourne DR, Altman DG, for the CONSORT Group. Consort 2010 statement: extension to cluster randomised trials. *BMJ.* 2012;345: e5661–e5661. doi:10.1136/bmj.e5661

9 Appendices

9.1 Annex A. Detailed schematic of study design

Figure A1. Detailed schematic of villages within split-plot randomized design



9.2 Annex B. Study power curves

Figure A2. Power curves for study designs with 12 HCCA

Note: Minimum power at 80%, with total number to be sampled per village

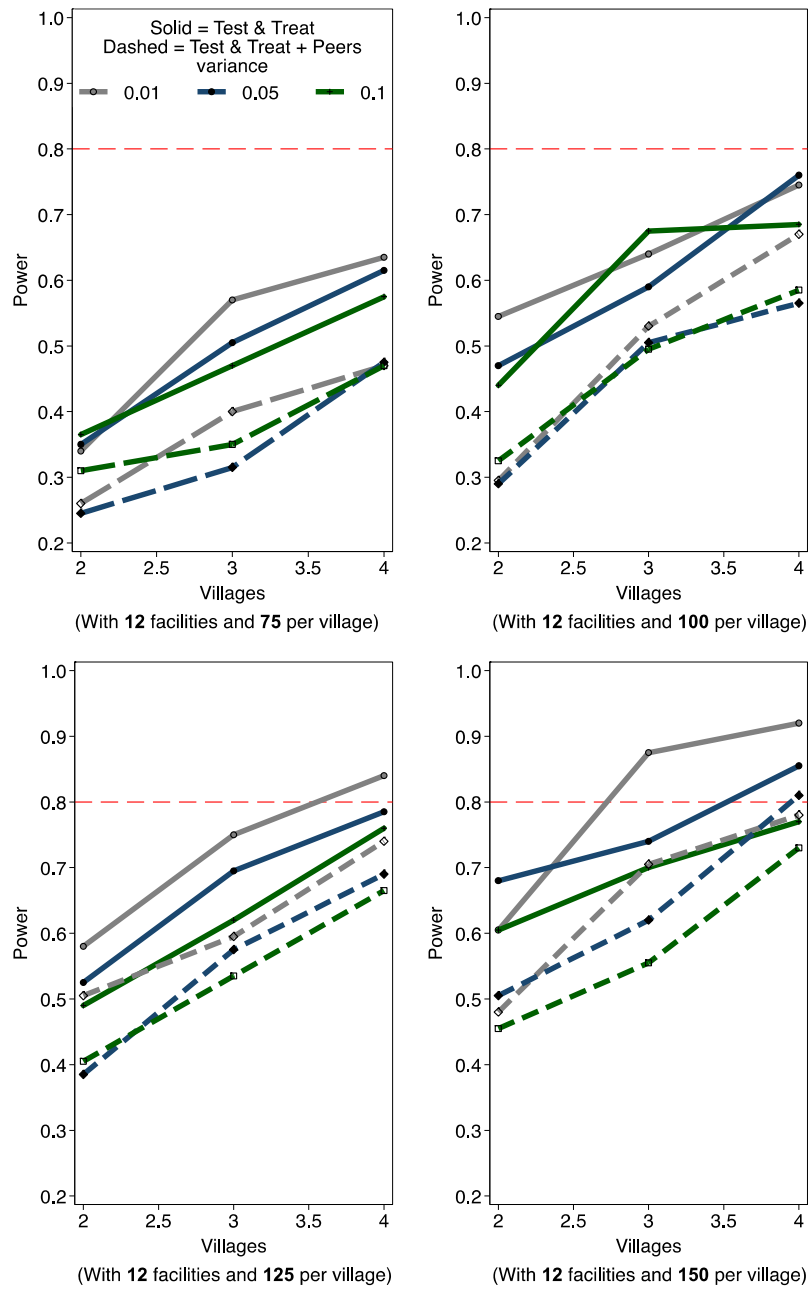
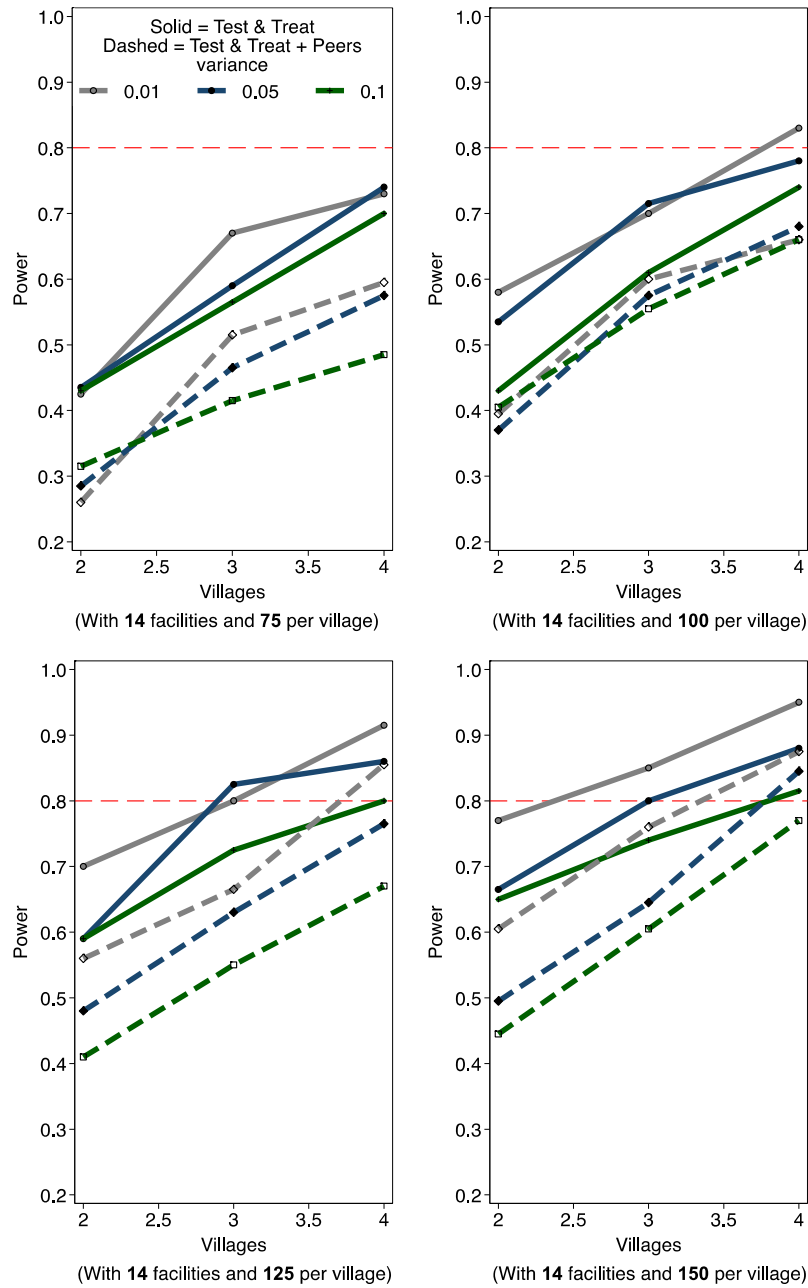


Figure A3. Power curves for study designs with 14 HCCA

Note: Minimum power at 80%, with total number to be sampled per village



- 9.3 Annex C. Cross-sectional survey informed consent form**
- 9.4 Annex D. Baseline/end-line household survey questionnaire**
- 9.5 Annex E. Individual blood collection informed consent (cross-sectional survey)**
- 9.6 Annex F. Blood collection results form (cross-sectional survey)**
- 9.7 Annex G. MTAT household survey informed consent form**
- 9.8 Annex H. MTAT household survey questionnaire**
- 9.9 Annex I. MTAT blood collection informed consent form**
- 9.10 Annex J. FTAT survey and blood collection informed consent form**
- 9.11 Annex K. FTAT HRP survey questionnaire**
- 9.12 Annex L. G6PD testing referral letter**
- 9.13 Annex M. G6PD health facility register form**
- 9.14 Annex N. Adverse event reporting form**
- 9.15 Annex O. Severe adverse event investigation form**
- 9.16 Annex P. FDG/KII informed consent form**
- 9.17 Annex Q. MTAT team FGD guide**
- 9.18 Annex R. Peer navigator FGD guide**
- 9.19 Annex S. Study team and district KII guide**
- 9.20 Annex T. Treatment adherence follow-up form**
- 9.21 Annex U. Peer navigator FTAT competency checklist**
- 9.22 Annex V. Peer navigator interview guide**
- 9.23 Annex W. HRP interview guide**